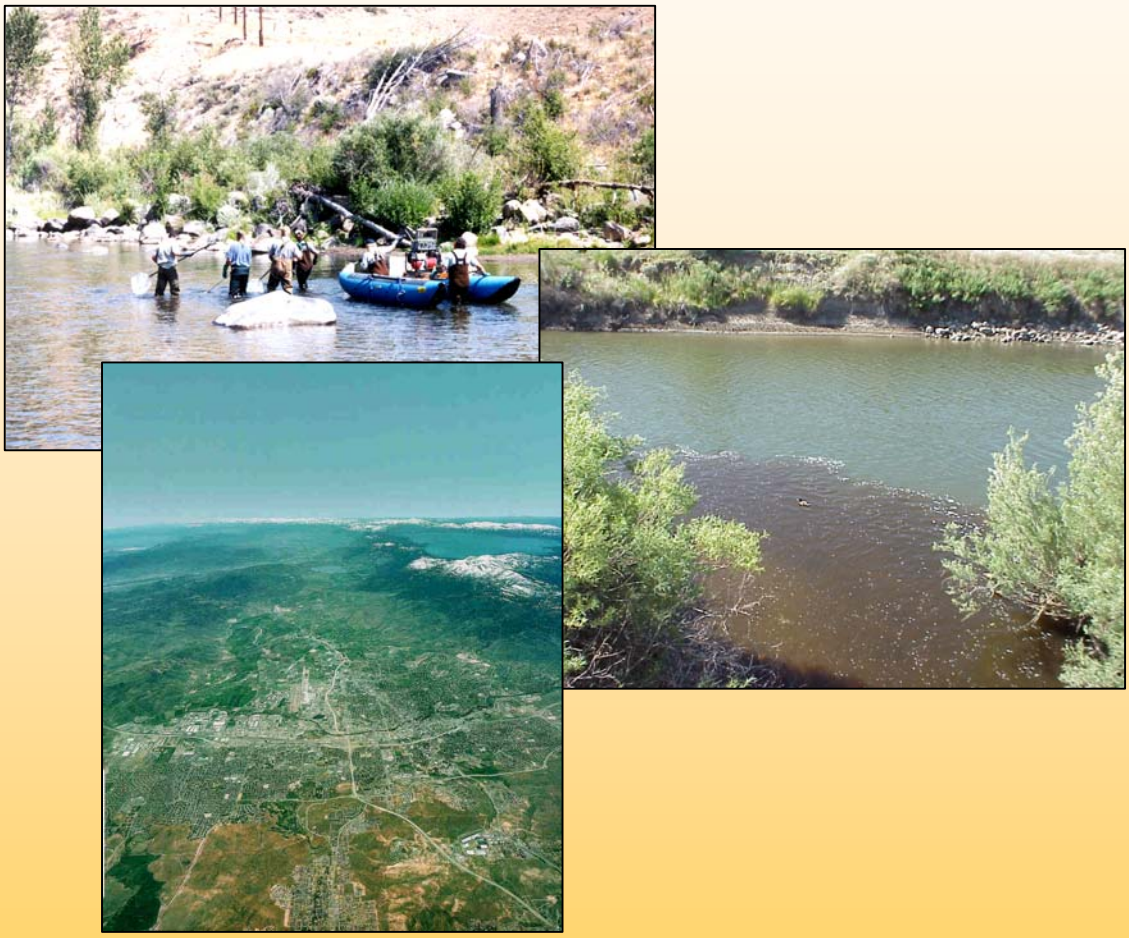
Preliminary Assessment of Contaminants and Potential Effects to Fish of the Truckee River, Nevada



U.S. Fish and Wildlife Service
Division of Environmental Quality
Nevada Fish and Wildlife Office
Reno, Nevada

A COORDINATED EFFORT

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Front cover photographs: Clockwise from top left, electroshocking fish in the Truckee River at Verdi, Nevada, August 2002, photograph by Timothy Rowe; North Truckee Drain confluence with the Truckee River, Sparks, Nevada, June 2000, photograph by William Cowan; aerial view of the Truckee Meadows and the Reno-Sparks urban area, 1993, photograph by Great Basin Aerial Surveys.

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Preliminary Assessment of Contaminants and Potential Effects to Fish of the Truckee River, Nevada

by

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EXECUTIVE SUMMARY

Previous investigations by U.S. Geological Survey (USGS) and others reported elevated concentrations of a variety of metals and polycyclic aromatic hydrocarbons (PAH) in Truckee River sediment collected in and downstream of the Reno-Sparks metropolitan area in Nevada in 1998. USGS scientists also documented elevated contaminant concentrations in fish and aquatic invertebrates which exceeded published biological effects criteria. In 1999 U.S. Fish and Wildlife Service (Service) biologists also noted a higher incidence of lesions, hemorrhagic septicemia, and external parasites in fish collected in this same reach. Therefore, the Service initiated a synoptic investigation in 2002 to determine if contaminants are affecting or have the potential to affect fish health, survival, or reproductive potential in the lower Truckee River. Specific Service objectives included: 1) evaluation of fish abundance and community structure; 2) assessment of the external condition of fish; 3) detailed evaluation of salmonid health (i.e., internal/external condition, histology, cytology, disease, and parasites); 4) characterization of fish contaminant exposure and accumulation; and 5) screening for indicators of endocrine disruption.

Fish were collected from 5 sampling sites on the Truckee River from Verdi, Nevada to its terminus near the Marble Bluff Dam at Pyramid Lake. Abundance and community structure values (species evenness and Index of Biotic Integrity) declined in a downstream fashion with notable reductions occurring at the Lockwood and Marble Bluff sample sites which were likely a result of cumulative effects of urbanization, loss of riparian cover, reduced flows, increased water temperature, as well as contaminants. Condition of brown trout and mountain/Tahoe suckers were significantly reduced at downstream sites. High percentages of external anomalies were also observed at sampling sites downstream of the Reno-Sparks urban area and ranged from 11% at Marble Bluff to a maximum of 43% at Lockwood. These anomalies were also likely the result of non-point sources, sewage effluent discharges, and reduced flows.

Evaluations of salmonid health revealed no significant issues with regards to organosomatic assays, blood chemistry, microbiological assessment, and histological evaluation from each sampling site. However, some data indicated suspected infections of bacterial kidney disease and other bacterial-type infections. However, these infections were not expressive enough or had detrimental impacts to those fish.

To assess contaminant exposure and accumulation, five to seven trout of appropriate size (≥ 200 mm) were randomly selected from sampling sites and were analyzed for polycyclic aromatic hydrocarbon metabolites in bile and concentrations of metals or trace elements in whole fish. Bile data revealed fish were being exposed to elevated concentrations of naphthalene and phenanthrene in the Reno-Sparks area. These concentrations, which were likely the result of urban run-off sources, exceeded criteria considered as contaminated. Whole fish data revealed concentrations of aluminum, barium, iron, and manganese were highest in rainbow trout compared to brown trout. Mercury concentrations in brown trout did not exceed water quality standards established by the Pyramid Lake Paiute Tribe. Concentrations of aluminum and barium in whole fish were highest above Reno and were likely the result of geochemical interactions of stream water with specific bedrock types. However, none of these concentrations exceeded known adverse biological effects. Concentrations of arsenic, mercury, and selenium in

whole fish were highest at the Tracy sampling site located below the Reno-Sparks urban area. The sources of uptake for these constituents originate mostly from geothermal springs, historic mine wastes, irrigation, and tertiary-treated sewage effluent within the Steamboat Creek drainage. Arsenic and selenium concentrations did not exceed known adverse biological effects. Mercury concentrations in trout downstream of the Reno-Sparks urban area did not exceed avian dietary effects, fish consumption guidelines, and water quality standards established by the Pyramid Lake Paiute Tribe.

Several studies have associated municipal waste water discharges with endocrine system effects in fish. Because treated municipal waste water represents a significant component of flows in the lower Truckee River, blood plasma was collected to screen for indicators of endocrine disruption in trout. Vitellogenin (VTG) concentrations were detected in two males downstream of the Reno-sparks urban area. Male fish do not normally produce VTG, but the hepatic estrogen receptor and the gene that encodes for VTG is still present. The result is that when male fish are exposed to estrogenic compounds, VTG production can be induced. Also, all adult males in the fish health assessment had no mature testes at all sites. The presence of VTG in the two males combined with the organosomatic data provides some evidence of potential endocrine disruption in individual trout. However, additional research is needed to assess which endocrine disrupting compounds may be present in the Truckee River, and the extent to which these compounds may be affecting fish populations.

The long-term health and reproductive potential of fish in the Truckee River will be increasingly affected as the Reno-Sparks urban area continues to expand. Restoration of river function and augmentation of wetlands within the floodplain would assist in attenuating contributions of contaminants from various point and non-point sources. Improvements in sewage effluent discharges and effective urban planning can also assist to reduce both point and non-point sources of some contaminants. Without addressing these issues, these point and non-point sources of contaminants will present significant challenges to maintaining a healthy fishery and prevent long-term restoration efforts of Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) in the Truckee River.

1.0 INTRODUCTION

In 1998, the U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA)Program reported elevated concentrations of a variety of metals and polycyclic aromatic hydrocarbons (PAH) in Truckee River sediment collected in and downstream of the Reno-Sparks metropolitan area in Nevada (Bevans et al. 1998). Contamination extended from Reno to at least 20 miles downstream at Tracy, Nevada. Metal and PAH concentrations were greater than the national 75th percentile reported by NAWQA and, in several instances, exceeded published biological effects criteria. NAWQA scientists also documented elevated contaminant concentrations in fish and aquatic invertebrates and noted a higher incidence of lesions, hemorrhagic septicemia, and external parasites in fish collected in this reach. In 1999, U.S. Fish and Wildlife Service (Service) biologists monitoring Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) (LCT) survival and stocking success in the lower Truckee River also reported a high incidence of external anomalies in fish from the reach of river downstream of Reno (William Cowan, Fishery Biologist, USFWS, Reno, NV, pers. comm. 2000). External anomalies again included external lesions, hemorrhagic septicemia, and external parasites.

Several potential sources of contaminants in lower Truckee River sediments have been identified. Elevated contaminant concentrations have been documented in permitted discharges to the river, including treated municipal waste water, commercial sump pumping discharges, and dewatering operations (Nevada Division of Environmental Protection file information, 2000). Under low flow conditions, these permitted discharges (particularly treated municipal waste water) account for the majority of flow in the lower Truckee River. Several non-point source discharges (stormwater runoff, urban runoff, agricultural return flows, and groundwater inflow) have also been identified as potential contaminant sources in the lower Truckee River. However, the relative contribution of contaminants in point and non-point source discharges is uncertain.

1.1 Purpose and Scope

The Service initiated a synoptic investigation in 2002 to determine if contaminants are affecting or have the potential to affect fish health, survival, or reproductive potential in the lower Truckee River. Specific Service objectives included: 1) evaluation of fish diversity, abundance, and community structure; 2) assessment of the external condition of fish; 3) detailed evaluation of salmonid health (i.e., internal/external condition, histology, cytology, disease, and parasites); 4) characterization of fish contaminant exposure and accumulation; and 5) screening for indicators of endocrine disruption.

1.2 Study Area

The Truckee River begins at the outlet of Lake Tahoe, California (altitude about 6,218 ft.) and flows for about 120 miles to Pyramid Lake, Nevada (a terminal lake, altitude about 3,790 ft; Brown et al. 1986) (fig. 1). Average annual precipitation in the upper part of the basin is nearly 30 inches (in.) while annual precipitation in the lower portion averages about 5

in. (Covay et al. 1996). Hydrology of the Truckee River Basin is characterized by cycles of flood and drought with snowmelt as the primary source of water. Peak discharge typically coincides with snowmelt in the spring, and the minimum discharge typically occurs in late summer. The Truckee River basin contains a complex mixture of historic mining, urban, and agricultural land uses. Information about the five sites sampled in this study, including potential anthropogenic sources of contaminants in each reach is provided in table 1.

Figure 1. Truckee River Basin in Nevada and California, with sampling sites identified.

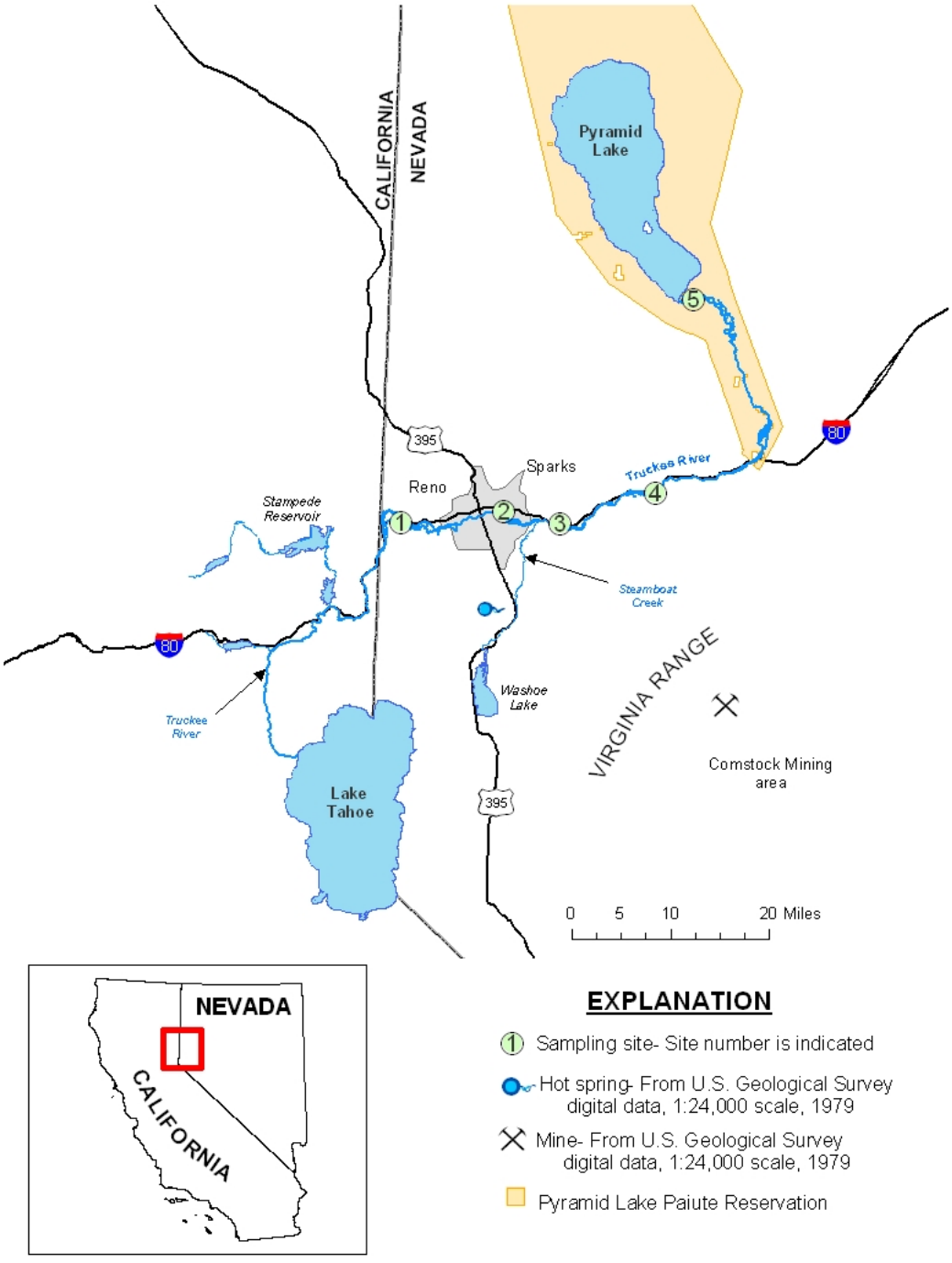


Table 1. Stream sites in the Truckee River where fish surveys were conducted and contaminant samples collected, August, 2002.

Site no. (fig. 1)	Site name	Distance from origin at Lake Tahoe outlet (river miles)	Principal anthropogenic sources of contaminants
1	Verdi	43	Federal highway; Tahoe City and Truckee, Calif.
2	Reno	58	Reno, Nev. urban area.
3	Lockwood	66	Reno-Sparks, Nev. urban area; tertiary treated sewage effluent; mine tailings.
4	Tracy	77	Reno-Sparks, Nev. urban area; tertiary treated sewage effluent; mine tailings.
5	Marble Bluff	120	Light agriculture; Reno-Sparks, Nev. urban area; Wadsworth, Nev.; tertiary treated sewage effluent; mine tailings.

Historic mining activity in the Truckee River Basin was primarily Comstock ore processing in the upper part of the Steamboat Creek drainage basin. This tributary enters the Truckee river at river mile 62.7 (Brown et al. 1986, p.107). Runoff from urban and agricultural activities is a potential source of contaminants in the Truckee River. A primary railroad route and two major highways also intersect and parallel the river. Until recently, urban runoff flowed unimpeded into the river. However, runoff detention ponds and best management practices are now required in areas of new construction. Tertiary-treated sewage effluent is discharged to the river, by way of Steamboat Creek, downstream of the cities of Reno and Sparks. The lower Truckee River within the Pyramid Lake Paiute Reservation also receives irrigation return flows from alfalfa fields.

2.0 METHODS

Each sampling site was electro-shocked with a pulsed DC electrical current to collect fish using techniques described by Kolz et al. (1995). Fish were captured using a cataraft towed barge electro-fishing unit composed of a Smith-Root Generator-Powered Pulsator (GPP) electro-fisher unit mounted on a plywood platform secured to the cataraft rowers frame and a 5 horsepower, 2.5 kilowatt gasoline Honda generator supplying the electrical output. The electrical field on the barge was generated by two anode probes containing 9-inch diameter rings and two cathode cables mounted along the flanks of the cataraft tubes. Operation required one electro-fisher operator who pushed the barge upstream, two anode probe operators, and several netters who flanked probe operators. Netters collected stunned fish and transported them in 5-gallon buckets to a streamside live car. Data collected from captured fish included the following components: 1) fish condition and community structure; 2) salmonid health; 3) contaminant exposure and accumulation in salmonids; and 4) determination of hormone levels in salmonids for

screening of endocrine disruption. A description of methods for each component is provided below.

2.1 Fish Community Structure and Condition

To describe and assess fish community structure, fish abundance, species evenness values, and Index of Biotic Integrity (IBI) scores were determined for each sampling site. Species evenness values, which describe the equitability of abundances among species in a community, were determined using the Pielou's J' index as described in Newman (1995). IBI methodologies provide a reproducible method to evaluate and rank relative condition of stream fish communities on a geographic scale and to assess changes over time (Miller et al. 1988; Plafkin et al. 1989). The IBI was first proposed by Karr (1981) to synthesize various aspects of fish assemblage condition into a single, easily understood number. Fish IBIs typically sum standardized scores of multiple metrics representing species richness; general tolerance; relative abundance; anomalies; and trophic, habitat, and reproductive guilds. For IBI analysis in this study, total IBI scores were determined for each sample site utilizing eleven metrics as described by Hughes et al. (2005). Each metric had a value continuously from zero to one as recommended by Ganasan and Hughes (1998), Hughes et al. (1998), and Minns et al. (1994). Mountain and Tahoe sucker species (*Catostomus platyrhynchus* and *Catostomus tahoensis*) were combined for analyses since they occupy the same ecological niche and physiology is mostly identical. The eleven metrics and their scoring criteria are provided in table 2.

Environmental stress can affect growth rate and general condition of fish. Condition factors, such as Fulton's condition factor, provide a relative measure of nutritional state or "well being" of individual fish and populations (Anderson and Gutreuter 1983). Such factors may also be used to compare relative condition of populations and to monitor environmental change over time (Ney 1993). To assess the fish health and general condition, fish of each species from each sampling site were measured, weighed, and assessed for indicators of disease, parasites, and external anomalies. Length and weight data were used to calculate Fulton's condition factor for each fish and the species for each sampling site using methods described in Anderson and Gutreuter (1983). Examination of external condition of fish was adapted from procedures provided in Meyer and Barclay (1990) and methods of external fish condition assessment provided in Foott (1990) and Goede and Barton (1987). Fish species, length, weight, and any abnormalities were recorded on a separate form for each site. All fish, with the exception of trout collected for health assessment or chemical analyses, were released back to the sampling site from which they were collected.

Table 2. Index of Biotic Integrity (IBI) metrics for fish in the Truckee River, as taken from Hughes et al. (2005).

Metric[a]	Scoring criteria	Total metric value
Number of native species	0 to ≥5 species present (0.2 points for each species)	0 to 1
Percent Paiute sculpin individuals	0 to ≥10% (0.1 points for each percent)	0 to 1
Percent mountain whitefish individuals	0 to ≥10% (0.1 points for each percent)	0 to 1
Evidence of reproduction by Paiute sculpin and/or mountain whitefish	0.5 added for each fish species	0 to 1
Percent Lahontan cutthroat trout individuals	0 to ≥10% (0.1 points for each percent)	0 to 1
Percent individuals that are sensitive species	0 to ≥20% (0.05 points for each percent)	0 to 1
Percent mountain sucker individuals	≥20 to 0% (-0.05 points for each percent)	1 to 0
Percent individuals that are omnivorous as adults	≥20 to 0% (-0.05 points for each percent)	1 to 0
Percent individuals that are generally tolerant	≥5 to 0% (-0.2 points for each percent)	1 to 0
Percent individuals those are alien to the Truckee River.	≥25 to 0% (-0.04 points for each percent)	1 to 0
Percent individuals with external anomalies	≥5 to 0% (-0.2 points for each percent)	1 to 0

[a] See Table 3 for scientific names of fish

2.2 Assessment of Salmonid Health

Up to 10 trout of appropriate size (200 - 300 mm) were sacrificed for organosomatic assays, blood chemistry, microbiological assessment, and histological evaluation from each sampling site. To prevent possible interference with contaminant assays, fish were euthanized by concussion rather than chemical anesthetics. Instruments and working surfaces used for dissections were cleaned with a brush and Citranox® detergent, rinsed with a dilute nitric acid solution, and triple rinsed with de-ionized water prior to use on each fish. Each fish was rapidly examined for external abnormalities, weighed and measured for both total and fork lengths. It was then bled from the caudal peduncle into heparinized, 5 mL Vacutainer® blood collection tubes. A subsample was removed for preparation of a blood smear, microhematocrit and leukocrit measurements, and plasma protein assay (Stoskopf 1993). The majority of the chilled blood sample was later centrifuged for plasma hormone assays. Plasma samples were held on dry ice. Blood smears were air dried, fixed in methanol, and stained by the Leishman–Giemsa method. A differential leukocyte count was performed on the first 100 white blood cells observed by light microscopy (Olympus BX40 Light Microscope 100x objective). Upon aseptic dissection, Brain Heart Infusion Agar was inoculated with kidney tissue and organs were rated for gross abnormalities. Bacterial cultures were held for 72 hrs and any isolates identified to genera or general group by standard microscopic and biochemical methods.

Liver and gonad were removed and weighted for hepatosomatic and gonadosomatic indices, respectively. Kidney samples were collected for *Renibacterium salmoninarum* antigen Enzyme-Linked Immunosorbant Assay (ELISA). Pooled samples of kidney and spleen were collected for viral assay on both EPC and CHSE214 cell lines. Cultures were held at 15°C for 16 days and examined for any cytopathic changes.

Kidney, liver, gill, spleen, and gonad were collected for histology. Histological samples were fixed in Davidson's fixative for 24 hrs, transferred to 70% ethanol, processed for 5 μm paraffin sections and stained with hematoxylin and eosin. The sections were examined for cellular abnormalities, semi-quantitative rating of endogenous pigments, and internal parasite infection.

2.3 Contaminant Exposure and Accumulation in Salmonids

To assess contaminant exposure and accumulation, five to seven trout of appropriate size (generally 200 - 300 mm) were randomly selected from the fish sacrificed for assessment of salmonid health (above) and samples were collected for contaminant analyses as described below.

2.3.1 Polycyclic Aromatic Hydrocarbon Metabolites

Upon opening of each fish, bile was extracted from the gallbladder with a sterile syringe and placed in a pre-cleaned 10 ml amber vial with a teflon-lined closure. Bile samples were refrigerated until determinations of polycyclic aromatic hydrocarbon (PAH) metabolite concentrations were made using High Performance Liquid

Chromatography (HPLC). Five µl of each sample/standard was injected directly onto the HPLC system using an autosampler. The response of the fluorescence detector was recorded with a HP-1000 computer for 35 minutes at naphthalene, phenanthrene and benzo[a]pyrene excitation/emission wavelength pairs. Peak areas were integrated for those peaks eluting between 5 and 28 minutes. These times were approximate and were based on times reported in the NOAA Technical Memorandum, NMFS F/NWC-102 and are verified for each system. Chromatographic conditions were selected so that no PAH metabolites eluted before 5 minutes and solvent contaminants eluted after 28 minutes for naphthalene, phenanthrene and benzo[a]pyrene metabolites. Phenanthrene and naphthalene peaks were identified from the calibration standards. The retention times were recorded and the areas of the reference standards were integrated. The mean response factor (ng/integration unit) was used to calculate sample analyte concentrations. The approximate retention times for naphthalene, phenanthrene, and benzo[a]pyrene were approximately 15, 17, and 21 minutes, respectively under the given analytical conditions. All concentrations of PAH metabolites in this study are reported in parts per million- wet weight.

2.3.2 Metals and Trace Elements in Whole Body

Whole fish carcasses assessed for fish health from each sampling site were placed in a labeled plastic bag and remained frozen until determinations of metal and trace element concentrations were made. Upon analysis, carcasses were homogenized with a meat grinder and then aliquots of approximately 100 grams were freeze-dried and further homogenized using a blender, or if necessary, a Spex mixer mill with a tungsten carbide vial and ball. After homogenization, each sample had percent moisture determined, wet digested with nitric acid, and converted into acidic digest solutions for analysis. Analyses included determination of mercury by cold vapor atomic adsorption spectroscopy, arsenic and selenium by hydride generation atomic adsorption, lead by graphite furnace atomic adsorption, and the remaining elements (aluminum, barium, cadmium, chromium, copper, iron, magnesium, manganese, strontium, and zinc) by inductively coupled plasma optical emission spectroscopy. All concentrations of metals and trace elements in this study are reported in parts per million- dry weight.

2.3.3 Analytical Data Quality Assessment

Laboratory analytical quality-assurance and quality-control (QA/QC) procedures were ensured by the Patuxent Analytical Control Facility (PACF) as described in their reference manual (PACF 1990). QA/QC procedures included the use of procedural blanks, duplicate samples, spiked samples, and reference materials. All samples met QA/QC requirements and were within standards.

2.4 Screening of Endocrine Disruption

Several studies have associated municipal waste water discharges with endocrine system effects in fish (Bevans et al. 1996, Flomar et al.1996, Harries et al. 1997). Because treated municipal waste water represents a significant component of flows in the lower Truckee River, we collected blood plasma to screen for indicators of endocrine disruption in trout. Up to 5 cubic centimeters (cc) of whole blood were collected from the caudal peduncle of up to10 trout of appropriate size (200 - 300 mm) from each sampling site. Blood collected was placed in lithium-heparinized vacutainer sample tubes and centrifuged at 10,000 RPM for 10 minutes from which plasma was decanted and placed in a 2 ml cryotubes. Blood plasma samples were frozen on dry ice and submitted to the U.S. Geological Survey Florida Carribean Science Center for analysis of sex steroid (17B-estradiol and 11-ketotestosterone) concentrations and ratios, concentrations of the synthetic hormone ethinyl estradiol, and vitellogenin concentrations. Hormone concentrations were determined by radioimmunoassay procedures. Vitellogenin concentrations were determined by enzyme-linked immunosorbent assay. Detailed procedures for these assays may be found in Goodbred et. al. (1997).

2.5 Data Analysis

All statistical analyses were conducted using SYSTAT® v.11 (SYSTAT Software Inc. 2004). Species evenness values and IBI scores were subjectively compared between sites since the values represented an aggregated function of data. Data for fish condition (Fulton factor), PAH concentrations in salmonid bile, metal/trace element concentrations in salmonid whole body, and concentrations of blood plasma hormones were transformed to ranks before statistical analyses.

As a result of mixed species, sexes, maturity levels and number of fish collected per site; statistical analysis was limited for assessment of salmonid health data. However, one-way ANOVA tests ($p \leq 0.05$) with a post-hoc, Bonferroni-adjusted, pairwise multiple comparison test were used to test for significant differences between sites.

Analytical data for bile, whole body, and blood plasma contained left-censored data; therefore, means for site, species, and sex were calculated using the Kaplan-Maier method described by Helsel (2005). If greater than 50 percent of an analyte's data in a matrix were below its detection limit, no Kaplan-Maier mean was calculated or additional statistical analysis performed. To explore potential biases in relationships between fish size and analytical results, Kruskal-Wallis tests ($p \leq 0.05$) were performed to determine if differences existed in fish sizes among sites, species, and sex. Relationships between fish weight and analytical results were determined using regression models. Differences in mean Fulton factors and analytical data (whole fish, bile, and hormones) were detected among sites, species, and sex (where applicable) using Kruskal-Wallis tests ($p \leq 0.05$). Mann-Whitney tests ($p \leq 0.05$) were used to identify specific matrices that were significantly different.

3.0 RESULTS AND DISCUSSION

3.1 Fish Community Structure and Condition

3.1.1 Fish Community Structure

Eleven fish species were captured among all five sites (table 3). The abundance of salmonids decreased in a downstream fashion with the most down-gradient sampling site at Marble Bluff having no salmonids (fig. 2). Lockwood and Marble Bluff sites were dominated by mountain/Tahoe suckers (Catostomids) and speckled dace respectively (fig. 3). Species evenness indices for each site ranged from a high of 1.05 at Tracy to a low of 0.44 at Lockwood (fig 4.).

Table 3. Fish species captured in the Truckee River and sampling sites where fish were collected, August 2002.

Common name	Scientific name	Site no. where collected (fig. 1)
Mountain sucker	*Catostomus platyrhynchus*	1, 2, 3, 4
Tahoe sucker	*Catostomus tahoensis*	1, 2, 3, 4, 5
Paiute sculpin	*Cottus beldingi*	1
Common carp	*Cyprinus carpio*	2
Mosquitofish	*Gambusia affinis*	5
Green sunfish	*Lepomis cyanellus*	5
Rainbow trout	*Oncorhynchus mykiss*	1, 2, 3, 4
Mountain whitefish	*Prosopium williamsoni*	1, 2, 3
Lahontan redside shiner	*Richardsonius egregius*	2, 3, 4, 5
Speckled dace	*Rhinichthys osculus*	1, 3, 4, 5
Brown trout	*Salmo trutta*	1, 2, 4

A gradual decline in fish community characteristics can be expected as changes in elevation, slope, and human disturbance begin to take effect in the watershed. However, the marked reductions of salmonid abundance and overall species evenness at the Lockwood and Marble Bluff sampling sites are presumed to result from significant anthropogenic alterations producing low quality physical and chemical habitat at those locations. The dominance of mountain and Tahoe suckers at the Lockwood site (84% of all individuals captured) reflects favorable habitat conditions and food resources for those species in this reach. These suckers are omnivorous bottom feeders that consume mostly algae and small benthic invertebrates (Moyle 2002). The nutrient-enriched effluent discharged directly upstream from the Truckee Meadows Water Reclamation Facility

(TMWRF) produces significant algal blooms in this reach, especially during summer periods under low flow conditions.

Principal anthropogenic alterations affecting the Lockwood and Marble Bluff sites are the discharge from TMWRF and water diversions from the river by several structures, most notably Derby Dam which can divert most of the flow in the Truckee River for agricultural purposes. The absence of salmonids in the lower portions of the river was also partly influenced by elevated water temperatures that averaged 23 degrees Celsius during the week of sampling. This temperature approached the upper limit for survival and growth of LCT (Dickerson and Vinyard 1999).

Figure 2. Abundance and type of salmonid individuals captured at sampling sites along the Truckee River, August 2002.

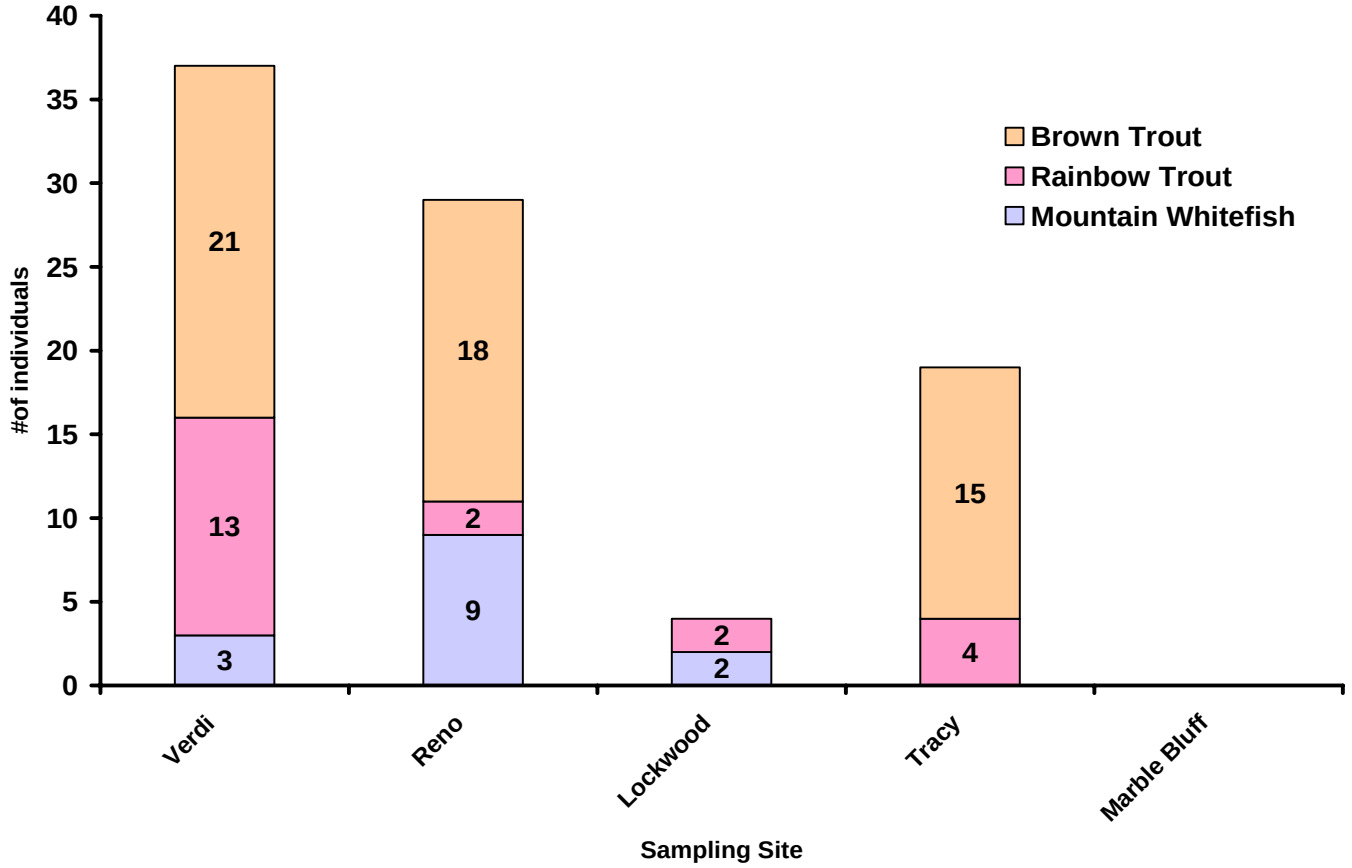


Figure 3. Percent composition of fish species captured at each sampling site on the Truckee River, August 2002.

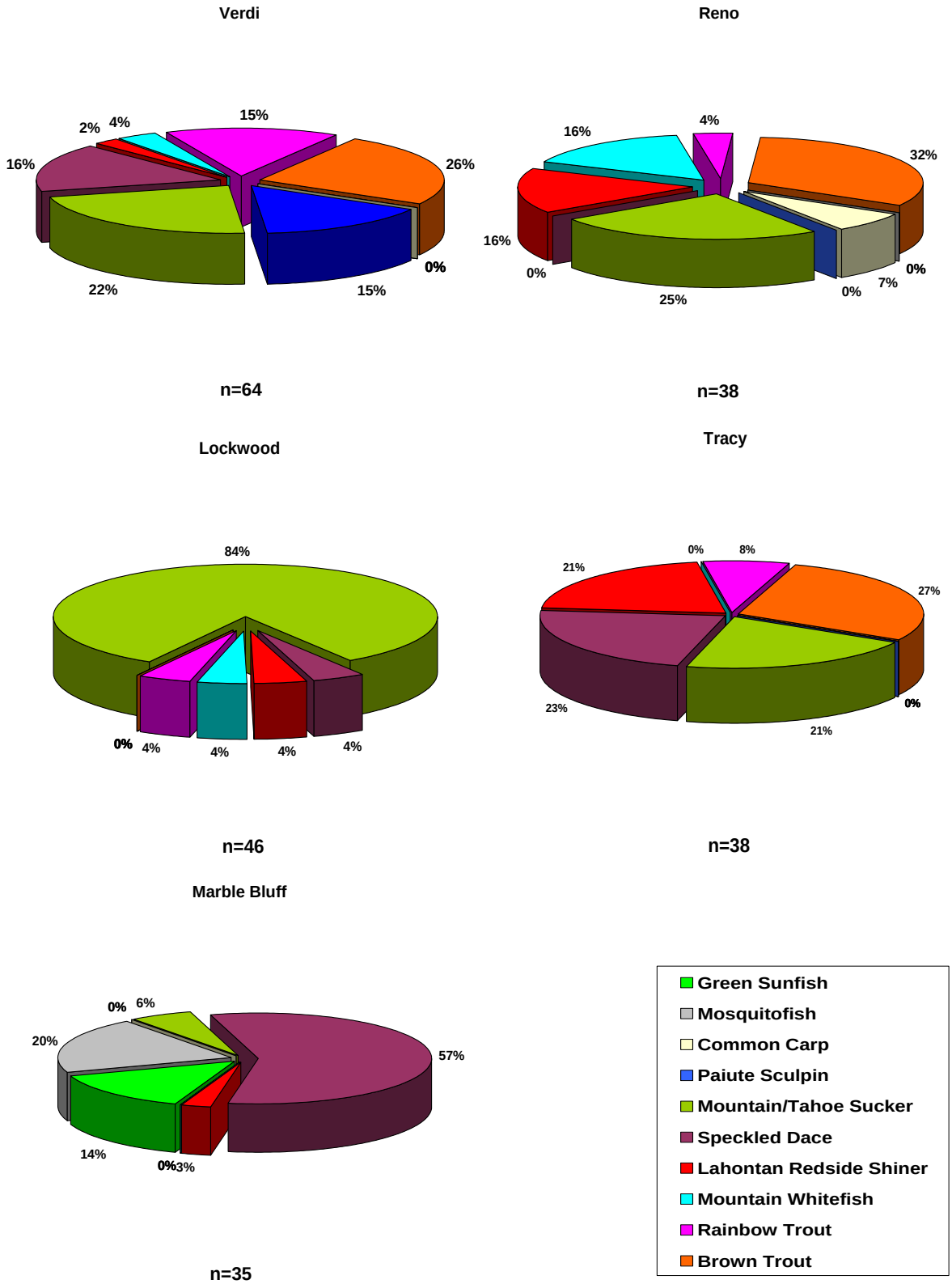
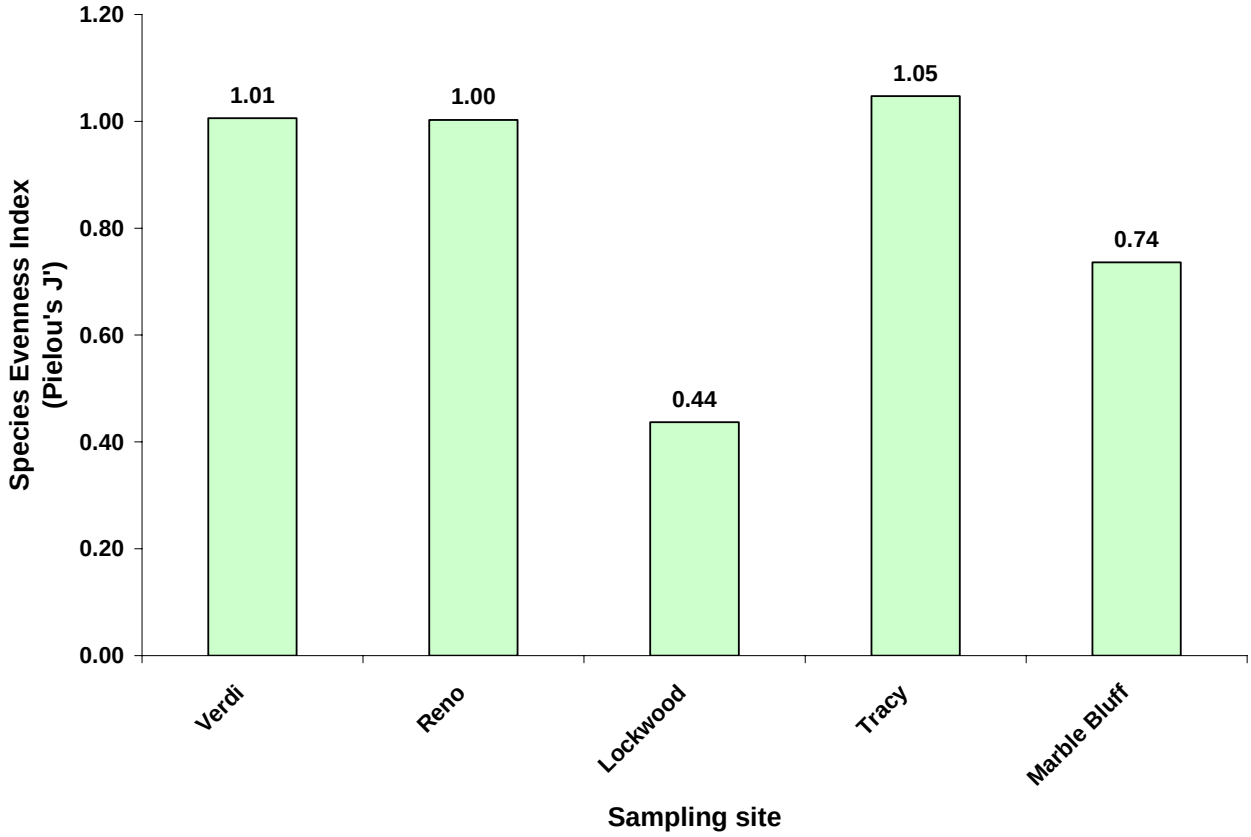


Figure 4. Species evenness indices (Pielou's J') for the fish community at each sampling site along the Truckee River, August 2002.

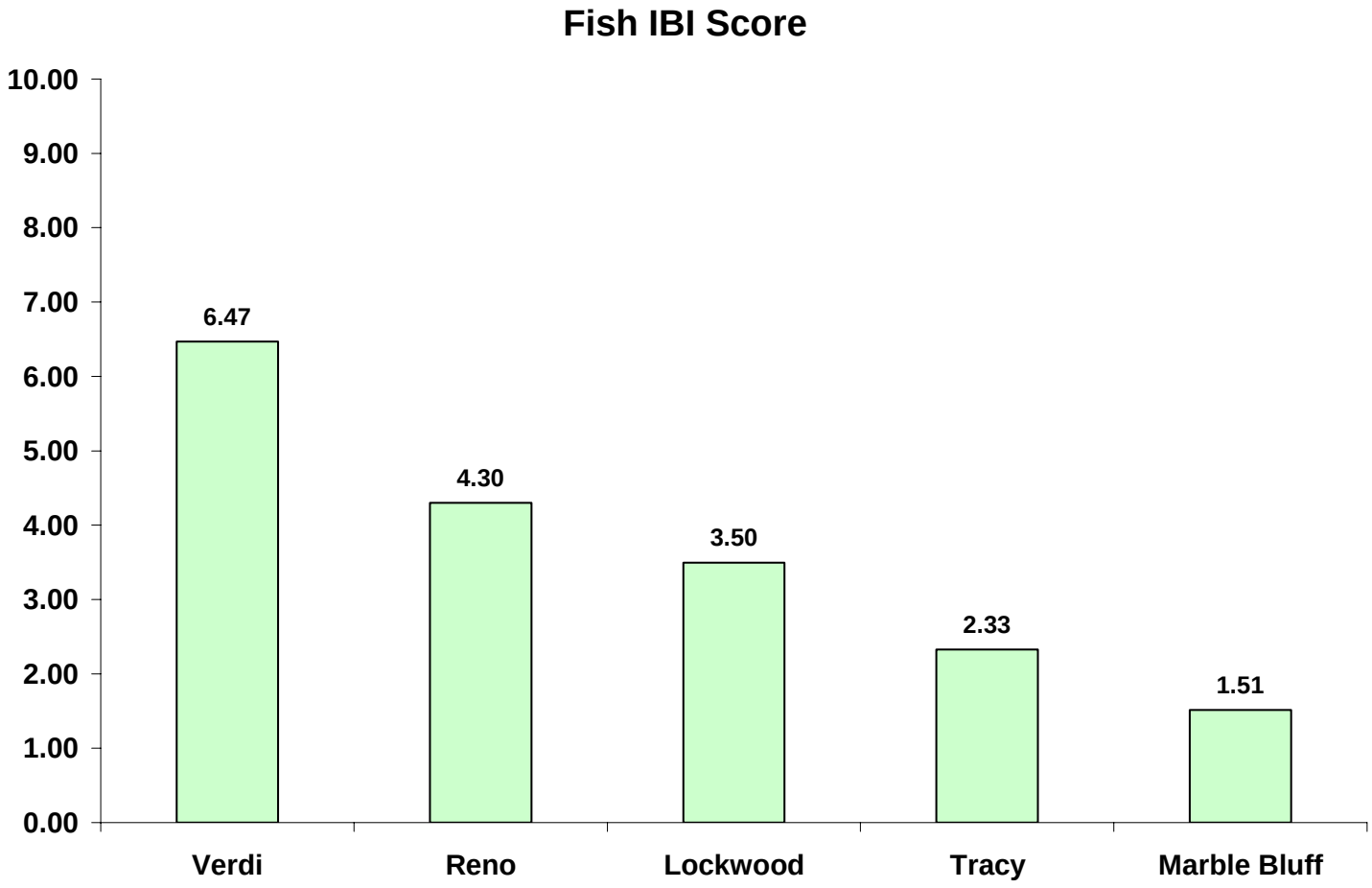


IBI scores for each sampling site consistently declined in a downstream fashion and ranged from a high of 6.47 at Verdi to a low of 1.51 at the its terminus near Marble Bluff Dam (fig. 5). Data used in the calculation of metrics and subsequent IBI scoring at each site is provided

Because IBI data from minimally-disturbed or natural reference sites are not available, quantitative estimates of reference conditions for the Truckee River are lacking. However, following interpretations of median IBI scores provided by Bozzetti and Schulz (2004), Hughes (1994), and Hughes et al. (1998), the Verdi sampling site was considered marginally impaired compared to the remaining sites downstream which were impaired. This has also been observed in other studies for rivers impacted by similar anthropogenic sources. Hughes and Gammon (1987) and Mebane et al. (2003) observed that IBI scores in the Willamette River, Oregon, decreased below urban outfalls. Increased urbanization of streams has also been associated with substantial decreases in IBI scores once catchment urbanization became greater than 5% (Steedman 1988; Wang et al. 1997, 2000; Klauda et al. 1998; Bryce and Hughes 2002; Snyder et al. 2003). Dams and reservoirs have also been associated with lower IBI scores (Lyons et al. 2001). Changes in IBI scores greater than 2 units over space or time are probably biologically significant (Hughes et al. 2005). Such was the case from Verdi to Reno. The reductions

in IBI scores for each site downgradient is likely the result of cumulative effects from wastewater effluent, multiple water diversions, and untreated agricultural return flows that increased nutrient, sediment, dissolved solids, and biocide loads**.**

Figure 5. Index of Biotic Integrity (IBI) scores for the fish community at sampling sites along the Truckee River, August 2002.



3.1.2 Fish Condition

Fulton condition factors revealed that significant differences existed among sites for brown trout, mountain whitefish, and mountain/Tahoe sucker (table 4). Mean brown trout condition factors were significantly lower at Tracy (0.98) compared to upstream sampling sites at Reno (1.11) and Verdi (1.10). Mountain/Tahoe sucker condition factors were significantly lower at Marble Bluff (0.79) compared to upstream sites at Tracy (1.22), Lockwood (1.15), Reno (1.17), and Verdi (1.20). Conversely, mountain whitefish condition factors were significantly higher at Lockwood (0.57) compared to upstream sites at Reno (0.512) and Verdi (0.473).

The lower Fulton condition values for brown trout and Catostomids at the downstream sites of Tracy and Marble Bluff, respectively, are likely a function of water quality, flow, and habitat conditions. Brown trout tend to occupy deeper, lower velocity and warmer waters than other trout; prey upon other species of fish; and compete with them for food and living space. Although brown trout are considered to be moderately tolerant of degraded water quality conditions, the potential reduction of food availability, increased water temperatures, and increased nutrients from TMWRF discharges are likely affecting the condition of brown trout in this reach of the river. The lower Fulton condition values for Catostomids at Marble Bluff may not be representative of that site since only two individuals were collected.

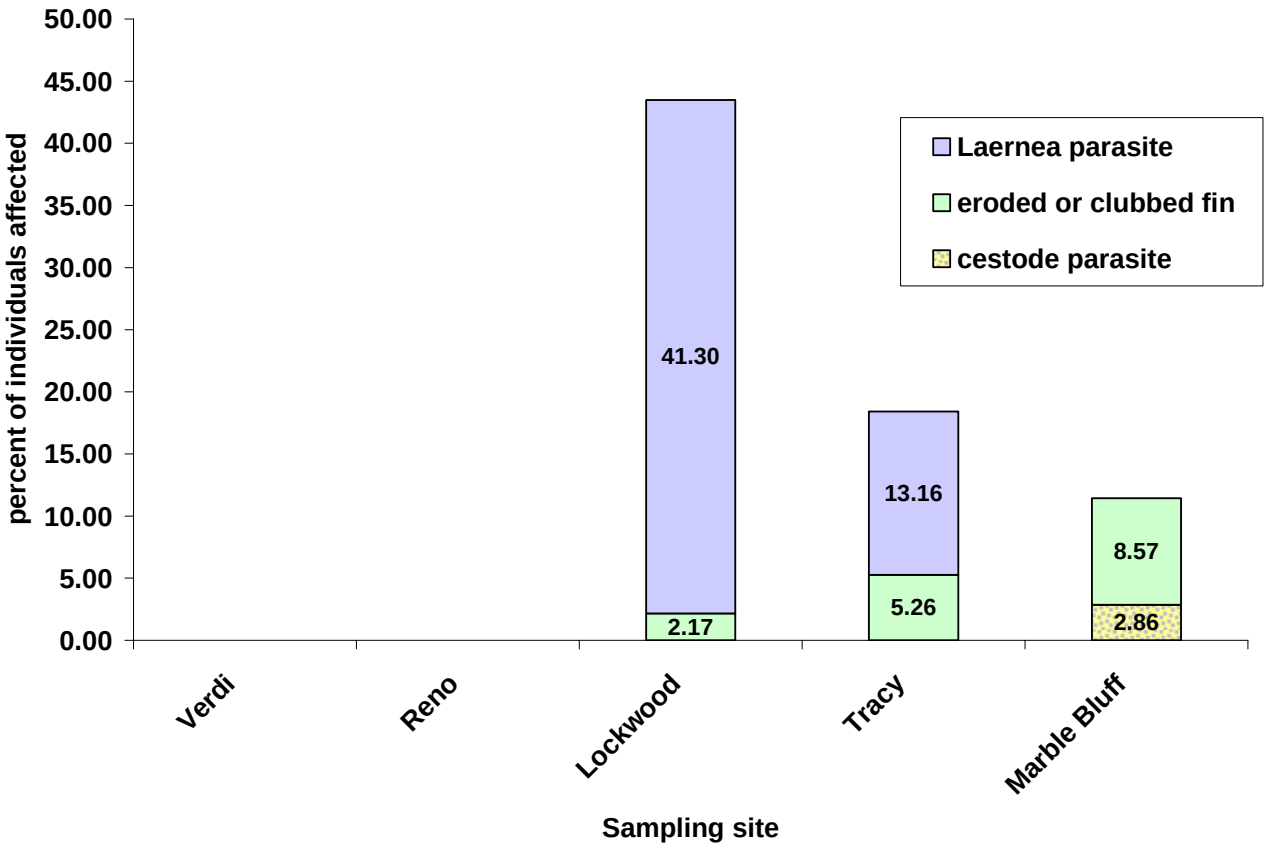
Table 4. Mean Fulton condition factors for fish species collected at three or more sampling sites on the Truckee River, August 2002.

Species	Verdi	Reno	Lockwood	Tracy	Marble Bluff
Mountain Whitefish	0.473 (a)	0.512 (a)	0.57 (b)	-	-
N	3	9	2		
Rainbow Trout	0.71	0.57	0.7	0.71	-
N	13	2	2	4	
Brown Trout	1.1 (a)	1.11 (a)	-	0.98 (b)	-
N	21	18		15	
Mountain/Tahoe Sucker	1.2 (a)	1.17 (a)	1.15 (a)	1.22 (a)	0.79 (b)
N	19	14	38	11	2
Speckled Dace	0.249	-	0.189	0.22	0.214
N	14		2	12	20
Lahontan Redside Shiner	2.98	2.63	2.63	2.49	1.88
N	2	9	2	11	1

- Letters indicate statistically significant differences

No external anomalies were observed on fish captured at the Reno or Verdi sampling sites. High percentages of external anomalies existed at sampling sites downstream of the Reno-Sparks urban area and ranged from 11% at Marble Bluff to a maximum of 43% at Lockwood (fig. 6). The majority of external anomalies observed were infestations of *Laernea spp.*, a crustacean parasite known commonly as "anchorworm". Other anomalies observed included a cestode parasite and clubbed or eroded fins. The increased prevalence of external parasites observed in fish collected in these reaches likely contributed to the reduced condition values observed. The high incidence of anchorworm infestations in Catostomids at the Lockwood site also provides evidence that degraded water quality conditions in the Truckee River can negatively affect fishes when favorable habitat and food resources exist.

Figure 6. Incidence of external anomalies on fish captured at sampling sites along the Truckee River, August 2002.



3.2 Assessment of Salmonid Health

3.2.1 Organosomatics

Two brown trout females at the Reno site had high gonadosomatic indices (GSI) of 8 and 11 %, respectively. All males examined had immature testes as indicated by gonadosomatic indices ≤ 1 (Table 5). When species were combined, no statistically significant differences in GSI was detected among sites for fish of the same sex (ANOVA, $p<0.05$). The Hepatosomatic indices (HIS) of male trout at Tracy were significantly higher than the males at Verdi (ANOVA, $p\leq 0.01$).

Table 5. Mean (Std. Dev.) data for hepatosomatic (HIS), and gonadosomatic (GSI) indices by sex for trout collected at sample sites on the Truckee River, August 2002.

	Verdi	**Reno**	**Tracy**
Female (no.)	3	8	3
HIS	0.661 (0.088) a	0.950 (0.410) a	1.348 (0.143) a
GSI	0.617 (0.272) a	4.27 (4.82) a	2.029 (0.467) a
Male (no.)	7	2	4
HIS	0.688 (0.256) b	0.725 (0.180) ab	1.519 (0.519) a
GSI	0.063 (0.201) a	0.033 a	0.237 (0.313) a

-Letters indicate statistically significant difference

Visceral Fat was rated on each sampled fish on a scale of 0 – 3, 0 = no visible visceral fat, 1 = some visible visceral fat (<50%), 2 = visceral fat (>50%) around pyloric caeca, and 3 = visceral fat (>90%) throughout the peritoneal cavity (table 6).

Table 6. Visceral fat scores for trout collected at sampling sites on the Truckee River, August, 2002.

	Verdi					Reno					Tracy				
Score	No. Fish	0	1	2	3	No. Fish	0	1	2	3	No. Fish	0	1	2	3
Total Rainbow trout	8					1					0				
Female	3		1	1	1	1			1		0				
Male	5		4	1		0					0				
Total Brown trout	2					9					7				
Female	0					7		1	5	1	3			3	
Male	2	1		1		2			2		4			3	1

All female trout contained gonadosomatic indices (GSI) within expected ranges. High GSI values in two brown trout females from the Reno sampling site were indicative of preparation for spawning in late fall or winter. However, all males had immature testes. Only one fish at the Verdi sampling site had primary spermatocytes within its testes with most specimens showing only spermatogonia. Immature testes would indicate either 1) maturation of testes in males of the Truckee River occurs in larger adults not observed by this study; or 2) maturation of male trout testes is being inhibited by hormonally active agents present in the Truckee River. The potential for hormonally active agents and the potential for endocrine disruption is provided later in the discussion of endocrine disruption screening.

3.2.2 Hematology

All fish examined appeared to have normal blood values (Miller et al. 1983). Accuracy of the plasma protein data was questionable, as samples were inadvertently held at room temperature overnight. Hematocrit (% packed erythrocyte volume), leukocrit (% packed white blood cell volume), and plasma protein (g/dL) data is given in ranges and is separated by the three (3) sites, species, and further by se. (table 7). The white blood cell profiles were normal for salmonids (Wedemeyer and Yasutake 1977) (table 8). There was some difficulty in distinguishing between activated thrombocytes (round form) and small lymphocytes. The low number of granulocytes (neutrophils) observed was in agreement with the low prevalence of microbial infection in all sampled groups.

Table 7. Blood data collected from trout at sampling sites along the Truckee River, August 2002. (NA- not applicable)

	Verdi	**Reno**	**Tracy**
Total rainbow trout	8	1	0
Female	3	1	0
Hematocrit Range (%)	30.77 – 53.97	52.38	NA
Leukocrit Range (%)	1.29 – 1.38	1.29	NA
Plasma Protein (g/dL)	4.4 – 4.6	5.2	NA
Male	5	0	0
Hematocrit Range (%)	18.33 – 47.54	NA	NA
Leukocrit Range (%)	0.93 – 1.52	NA	NA
Plasma Protein (g/dL)	3.9 – 4.8	NA	NA
Total brown trout	2	9	7
Female	0	7	3
Hematocrit Range (%)	NA	32.08 – 40.00	41.94 – 57.14
Leukocrit Range (%)	NA	0.62 – 1.80	1.94 – 3.00
Plasma Protein (g/dL)	NA	3.8 – 6.1	4.0 – 4.4
Male	2	2	4
Hematocrit Range (%)	39.68 – 43.94	32.00 – 44.62	34.38 – 65.00
Leukocrit Range (%)	1.05 – 1.24	0.65 – 1.32	1.36 – 3.75
Plasma Protein (g/dL)	4.1 – 5.8	3.1 – 4.7	3.3 – 4.6

Table 8. Mean values (+ or – SE) of white blood cell profiles from trout captured at sampling sites on the Truckee River, August 2002.

	N	Lymphocyte (%)	Thrombocyte (%)	Granulocytes (%)
Verdi	10			
$\bar{x}$		62.70 ± 24.34	39.20 ± 24.72	0.10 ± 0.32
Range		21 - 92	7 - 79	0 - 1
Reno	10			
$\bar{x}$		55.40 ± 22.87	45.40 ± 24.20	1.33 ± 3.04
Range		28 - 95	5 - 81	0 – 9
Tracy	7			
$\bar{x}$		73.43 ± 26.38	23.57 ± 28.08	0.14 ± 0.38
Range		26 - 100	0 - 73	0 - 1

3.2.3 Microbiological Assays

No bacteria were isolated from fish at the Verdi site. Motile gram-negative rod bacteria, belonging to the motile aeromonad group (i.e, *Aeromonas hydrophila, Pseudomonas sp.*), were isolated from 2 of 10 trout from the Reno site and 1 of 7 trout at the Tracy site. Although diseases associated with motile aeromonads are most severe among fish that are propagated under conditions of intensive culture, these bacteria may also affect feral fish and are common in the intestinal flora of apparently healthy fish (Trust et al. 1974). The bacterium is ubiquitous and occurs in most fresh water environments. It can be found both in the water column and in the top centimeter of sediment (Hazen 1979). Motile aeromonads are adapted to environments that have a wide range of conductivity, turbidity, pH, salinity, and temperature (Hazen et al. 1978).

No viruses were detected from any of the sampled fish. One kidney sample out of 27 fish showed a high antigen concentration for *Renibacterium salmoninarum*. *R. salmoninarum* is the causative agent of Bacterial Kidney Disease (BKD) and is an obligate pathogen of Salmonid fishes worldwide (Evelyn 1993). BKD is a chronic disease characterized by granulomatous lesions in the kidney and is frequently fatal. *R. salmoninarum* is a small (0.3-0.1 μm by 1.0-1.5 μm), nonmotile, nonspore-forming, nonacid-fast, Gram-positive diplobacillus (Fryer and Sanders 1981). It typically causes a slowly progressing systemic infection, with overt disease rarely evident until fish are 6-12 months old (Evelyn 1993). Fish with severe *R. salmoninarum* infections may show no obvious external signs, or may exhibit one or more of the following: lethargy; skin darkening; abdominal distension due to ascites; pale gills associated with anaemia; exophthalmos; haemorrhages around the vent; and cystic cavities in the skeletal muscle (World Organization for Animal Health 2003). Transmission can be both horizontal (fish-fish) and vertical (female-progeny). However, no clinical signs of BKD were seen in this individual fish. Six other kidney samples had low *R. salmoninarum* antigen values and were considered subclinically infected or potential latent carriers (1 = Verdi, 4 = Reno, and 1 = Tracy).

3.2.4 Histology

No significant abnormalities or parasite infections were observed in sections of liver, kidney, spleen, testes or gill from any of the sampled trout. The kidney sections had varying degrees of melanomacrophage aggregates in the interstitum. These aggregates also contained small quantities of hemosiderin (iron from recycled erythrocytes) and lipofuscin ("age") pigments. Moderate quantities of these endogenous pigments are normal in adult fish (J. Scott Foote, Fish Health Specialist, USFWS, Anderson, CA, pers. comm. 2004). No trend by sample location was obvious in pigment quantity or type.

3.3 Contaminant Exposure and Accumulation in Salmonids

3.3.1 Polycyclic Aromatic Hydrocarbon Metabolites

Results of PAH metabolite concentrations for all fish bile samples are provided in appendix 1. Kaplan-Maier mean concentrations of PAH metabolites for species, sexes, and sites are provided in table 9.

Individual concentrations of benzo(a)pyrene ranged from <0.05 to 0.6 ppm. Mean concentration for each sample site increased in a downstream fashion from a minimum of 0.24 ppm at Verdi to a maximum of 0.43 ppm at Tracy; however, means were not considered to be significantly different among sites, species, or sexes. Individual concentrations of naphthalene ranged from 3.1 to 190 ppm. No differences were detected between species or sexes. However, differences did exist among sites. The mean concentration for the Reno sampling site was significantly higher (130.75 ppm) than both the Verdi (53 ppm) and Tracy (75.02 ppm) sampling sites. Individual concentrations of phenanthrene ranged from 1.5 to 74 ppm. No differences were detected in concentrations between species or sexes. However, like naphthalene, the mean concentration at the Reno sampling site was significantly higher (48.25 ppm) than both the Verdi (17.08 ppm) and Tracy (29.9 ppm) sampling sites.

Aromatic hydrocarbons are potential carcinogens, and have been shown to adversely impact growth, reproduction, and survival (Arkoosh et al. 1994; Baumann 1984; Couch et al. 1983; Couch and Harshbarger 1985; Hendricks et al. 1985; Schultz and Schultz 1982). Tjeerdema and Crosby evaluated the bioconcentration and metabolic fate of naphthalene in Delta striped bass (*Morone saxatilis)* in 1993. They found that the bass rapidly accumulated naphthalene, with a 24-hr BCF of 283.7, and slowly depurated it. The skin contained the greatest fraction of the retained naphthalene residues (44.5%), however, when the bass where removed from the contaminated test chambers and allowed to depurate, concentrations sequestered in the viscera/gonads actually increased when all other tissue levels significantly declined. The potential for naphthalene to act a reproductive toxicant to other types of fish is unclear, but as a cumulative stressor to an already stressed fish, there may be an impact. The ability of trout to metabolize naphthalene to a more hydrophilic compound, and thus increase its excretion efficiency is also unknown, but if Phase I oxidation activity is low or non-inducible in salmonid gonads, it could contribute to an even greater accumulation in the reproductive tissues.

Table 9. Mean concentrations of polycyclic aromatic hydrocarbon metabolites (parts per million; wet weight) determined in salmonids among species, sex, and sampling sites in the Truckee River, August 2002.

Matrix		N	PAH metabolite		
			benzo(a)pyrene	naphthalene	phenanthrene
Species					
	Brown trout	10	0.37	94.71	35.75
	Rainbow trout	4	0.23	54.00	17.60
Sex					
	Female	9	0.34	76.46	28.43
	Male	5	0.30	95.00	34.40
Sampling site					
	Verdi	5	0.24	53.00	17.08
	Reno	4	0.33	130.75	48.25
	Tracy	5	0.43	75.02	29.90

The source of elevated sediment PAH concentrations determined for the reach of the Truckee River in and downstream of downtown Reno was identified by Bevans et al. (1998) as urban run-off. The higher concentrations of naphthalene and phenanthrene metabolites in trout bile at the Reno site indicated that fish within the Reno-Sparks urban area exhibited greater uptake and accumulation of PAH compared to other sites. Naphthalene and phenanthrene are lighter molecular weight compounds; therefore, accumulation and metabolization of lightweight PAH occurs more readily compared to other compounds such as benzo(a)pyrene. Trout from the Reno and Tracy sampling sites also exceeded the contamination criteria for naphthalene (60 to 80 ppm, wet weight) recommended by Cormier et al. (2000).

3.3.2 Metals and Trace Elements in Whole Fish

All thirteen metals and trace elements were detected in at least one individual whole fish sample at every sampling site. Results of metal and trace elements from whole fish samples collected in this study are provided in appendix 2. Results of mean metal and trace element concentrations among the various matrices are provided in table 10.

Many metals and trace elements are essential for health, forming integral components of proteins involved in all aspects of biological function. However, in excess these metals and trace elements are potentially toxic, and to maintain metal homeostasis organisms must tightly coordinate metal acquisition and excretion. The diet is the main source for essential metals, but in aquatic organisms an alternative uptake route is available from the water. Differences in uptake, retention, and excretion of metals and trace elements in fish are attributable to several factors, among which include a fish's trophic status and/or physiological state, and a metal or trace element's environmental availability (Bury et al 2003). With that respect, discussion on the metals and trace

elements that were determined to be significantly different among sex, species, and sites is provided in the sections below.

Table 10. Mean concentrations of metals and trace elements (parts per million; dry weight) determined in salmonids among sampling sites, species, and sexes for the Truckee River, August 2002 (BDL= sample results were below analytical detection limits in 50% or more of the matrix of interest; detection limits provided in appendix 2).

Analyte	MATRIX						
	Sampling site			*Species*		*Sex*	
	Verdi	**Reno**	**Tracy**	**Rainbow Trout**	**Brown Trout**	**Male**	**Female**
Aluminum	51.286	6.833	33.4	59.5	17.5	27.6	36.375
Arsenic	0.587	0.383	1.196	0.685	0.698	0.478	0.914
Boron	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Barium	3.441	1.035	1.112	3.883	1.047	2.221	1.706
Beryllium	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Cadmium	0.057	0.255	0.22	0.075	0.19	0.1	0.179
Chromium	0.65	1.525	0.56	0.75	1.017	1.09	0.775
Copper	9.1	9.017	7.72	9.8	8.133	8.17	9.338
Iron	110.143	44.167	62.2	124.833	49.833	65.9	86
Mercury	0.231	0.407	0.486	0.237	0.53	0.371	0.347
Magnesium	1120.429	1135	1174	1144	1138.25	1117.4	1168.625
Manganese	6.686	4.467	4.6	7.283	4.408	4.512	6.05
Molybdenum	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Lead	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Selenium	0.671	0.717	1.18	0.717	0.883	0.73	0.95
Strontium	27.514	29.433	20.78	28.467	25.192	20.65	30.79
Vanadium	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*	*BDL*
Zinc	119.129	121.33	152.8	126.95	130.35	125.47	133.9

Figure 7. Mean concentrations of metals and trace elements determined to be significantly different between rainbow and brown trout species collected from the Truckee River, August 2002.

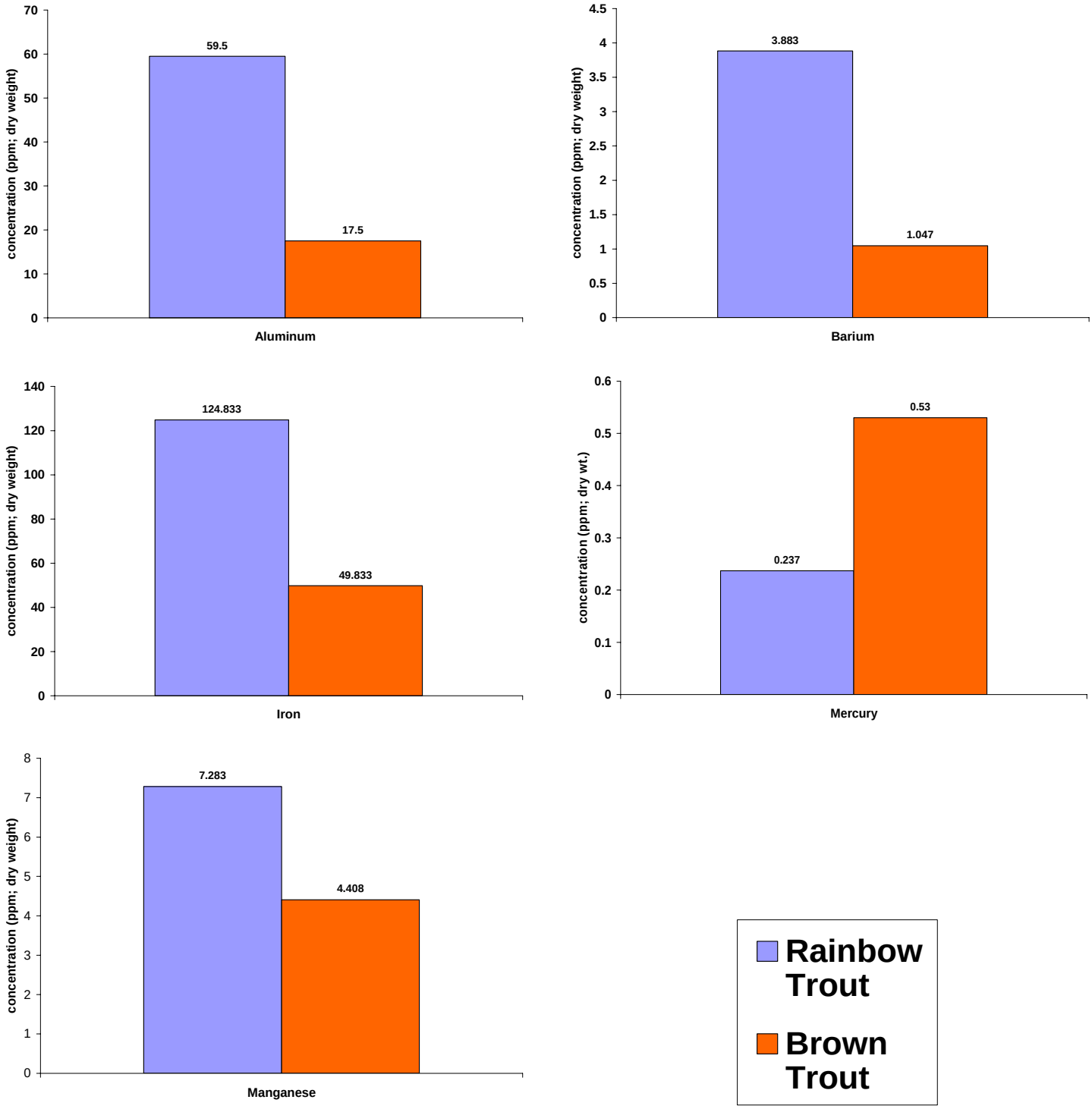
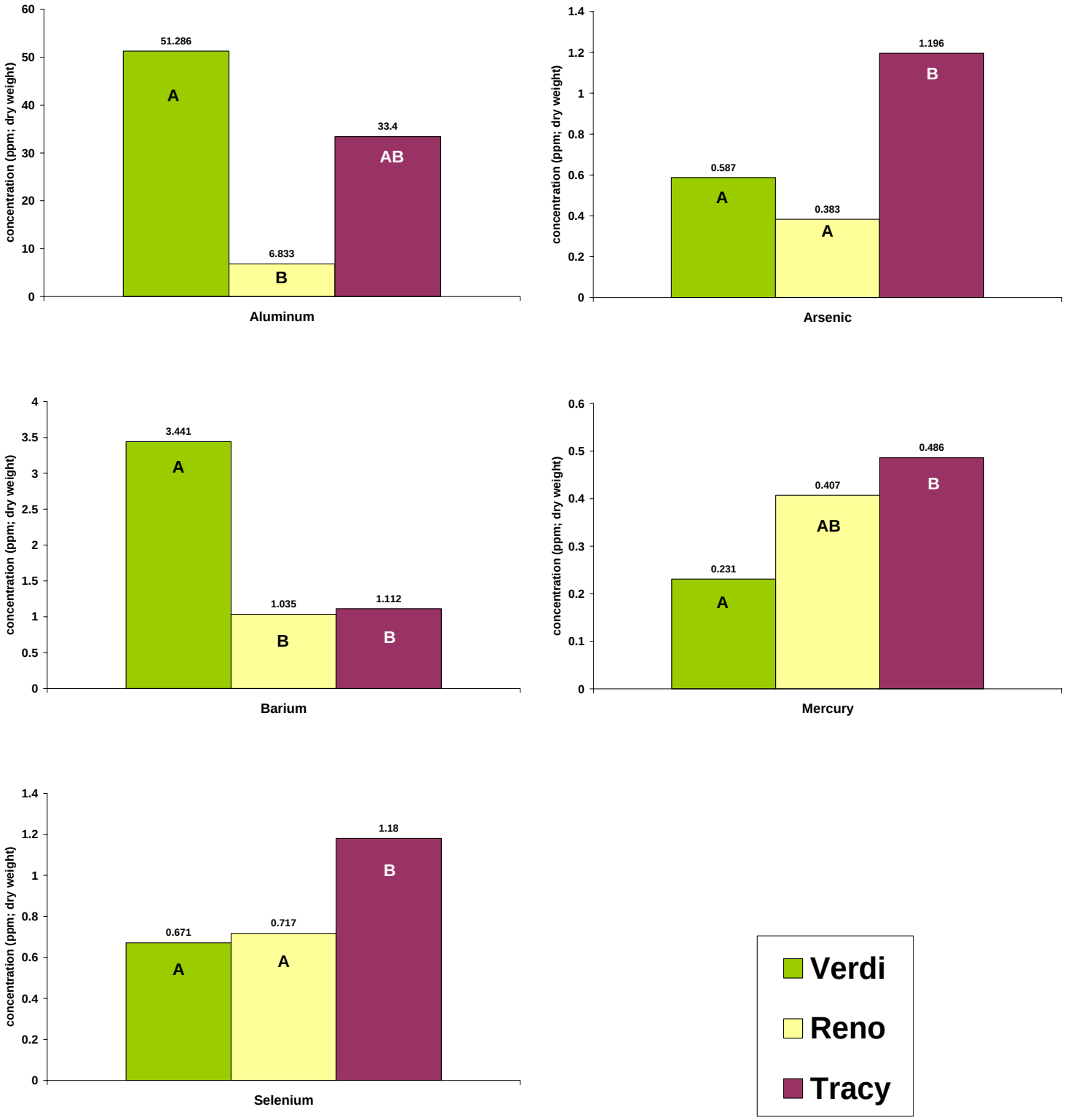


Figure 8. Mean concentrations of metals and trace elements significantly different between sampling sites on the Truckee River, August 2002 (letters indicate differences).



3.3.2.1 Sex Differences

Analyses of mean concentrations between male and female trout revealed manganese and strontium were significantly higher in females versus males. The mean manganese concentration for females was 6.05 ppm compared to 4.51 ppm for males. The mean strontium concentration for females was 30.79 ppm compared to 20.65 ppm for males.

Both of these elements have some function or impact in bone structure and development for vertebrates. Manganese is an essential nutrient that plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and the formation of glycosaminoglycans (Wedler 1994).

Strontium is chemically similar to calcium, with both elements being found in Group 2 of the periodic table and forming divalent cations. However, since strontium is not the same size as calcium, it does not substitute precisely for calcium in biological processes. Because of its similarity to calcium, strontium accumulates to a high degree in bone, and, in high concentrations, may seriously interfere with the normal process of bone development (ATDSR 2004). The increased concentrations for these two constituents are likely the result of increased mineral uptake functions from metabolic processes unique for female homeostasis. However, further information would be needed to verify these observations.

3.3.2.2 Species Differences

Analyses of mean concentrations between brown and rainbow trout species revealed significant differences for aluminum, barium, iron, mercury, and manganese. Results for species differences are presented in figure 9. Aluminum, barium, iron, and manganese concentrations were higher in rainbow trout. Mercury concentrations were higher in brown trout. Discussion on the reasons for these differences among species is provided below.

<u>Brown Trout</u>

Mercury bioconcentrates, bioaccumulates, and biomagnifies in aquatic food chains. These processes result in mercury residues in fish that are much higher than concentrations in the water in which they live, thereby providing an enriched contaminant source for piscivorous avian and mammalian wildlife. The higher concentrations of mercury observed in brown trout compared to rainbow trout result from the higher trophic status of brown trout that feeds predominantly on other fish versus the drift feeding strategies employed by rainbow trout. In terms of adverse effects to fish, the toxicological significance of body burdens is unknown, however, toxicity studies on fish have reported inhibition of reproduction, respiratory impairment, disruption of the osmoregulatory function of the gills (Burton et al. 1972; Evans 1987), reduction of monoamine and cholinesterase activities in neural tissue (Kirubagaran and Joy, 1990; Shaw and Panigrahi, 1990), reduction of acid and alkaline phosphatase activity responsible for liver and kidney membrane transport (Hinton and Koenig, 1975; Lakshmi

et al. 1991), adverse effects on liver and muscle protein synthesis (Nicholls et al. 1989), and disruption of a number of other essential biochemical processes, all associated with mercury exposure in fish. Beckvar et al. (2005) evaluated different approaches for deriving protective tissue residue effect concentrations for mercury in fish and determined a tissue-threshold effect level (t-TEL) of 0.2 ppm (wet weight) to be protective of sub-lethal endpoints (growth, reproduction, development, and behavior). All fish collected in this investigation were below this t-TEL with the exception of one individual.

For avian dietary effects, mercury concentrations in brown trout at the Tracy site did not exceed reproductive and behavioral effects determined for loons at 0.3 ppm, wet weight (Barr 1986). For human health effects, nearly all mercury in fish is in the more toxic methyl form (Wiener and Spry 1996). Therefore, the measurement of total mercury alone in fish is sufficient for evaluating mercury risk (U.S. Department of the Interior 1998). Brown trout at the Reno and Tracy sampling sites also did not exceed the U.S. Environmental Protection Agency (EPA) (2001) tissue residue criterion and the Pyramid Lake Paiute Tribe (2005) water quality standard of 0.3 and 0.271 ppm (wet weight), respectively.

Rainbow Trout

The significantly higher concentrations of aluminum, barium, iron, and manganese in rainbow trout suggest that behavioral or metabolic differences played a role in uptake and accumulation rates. However, the majority of rainbow trout analyzed for metal and trace elements in this study were taken from the Verdi site and may have biased results for constituents that were actually reflective of site differences. Comparisons of these four constituents to analyses of site differences revealed that aluminum and barium were likely reflective of site differences whereas iron and manganese were reflective of differences in species uptake. A review for the latter two is provided below.

Iron

Iron is an essential nutrient to almost all organisms. Iron positioning in the haem moiety of hemoglobin increases oxygen binding and carrying capacity, enabling oxygen transfer to all tissues in multicellular organisms. One of iron's key cellular functions is to confer redox activity to the cytochromes involved in respiration, due to its ability to exchange electrons in aerobic conditions (Bury et al. 2003). A negative consequence of iron's redox flexibility is that it produces oxygen free radicals that are toxic to the cell (Bury et al 2003). Consequently, in excess, iron can be detrimental to health. In addition, excess waterborne iron may be toxic to fish, due to the formation of iron `flocs' on the gills, resulting in gill clogging and respiratory perturbations (Peuranen et al. 1994; Dalzell and MacFarlane 1999). Observations of gills revealed no signs of osmoregulatory impairment.

The iron content of fish is, in general, considerably lower than that of other vertebrates (Van Dijk et al. 1975), but the precise daily iron requirements for fish are at present unknown. Aside from the generally lower levels of iron, it is widely assumed that iron metabolism and function in teleost fish is similar to that in other vertebrates (Lall 1989). Animals lose iron through defecation and epithelial sloughing, and this loss is compensated for by absorption from the diet. In fact, the

regulation of iron homeostasis is governed by intestinal absorption, as a regulated excretory mechanism is not known for iron in higher vertebrates (Andrews 2000). Therefore, increased concentrations of iron observed in rainbow trout is likely a function of specific accumulation from invertebrates in their diet.

Manganese

The transport and partitioning of manganese in water is controlled by the solubility of the specific chemical form present, which in turn is determined by pH, Eh (oxidation-reduction potential), and the characteristics of the available anions. The metal may exist in water in any of four oxidation states. Manganese (II) predominates in most waters (pH 4–7) but may become oxidized at a pH >8 or 9 (EPA 1984). The principal anion associated with Mn(II) in water is usually carbonate (CO_3^{-2}), and the concentration of manganese is limited by the relatively low solubility (65 mg/L) of $MnCO_3$ (Schaanning et al. 1988). Manganese in water may be significantly bioconcentrated at lower trophic levels. Folsom et al. (1963) estimated that the bioconcentration factor (BCF) of manganese was 2,500–6,300 for phytoplankton, 300–5,500 for marine algae, 800–830 for intertidal mussels, and 35–930 for coastal fish. Similarly, Thompson et al. (1972) estimated that the BCF of manganese was 100–600 for fish. Although information is limited on adverse effects to salmonids from manganese, the concentrations determined in rainbow trout by this study would suggest a low risk from bioaccumulation.

3.3.2.3 Site Differences

Analyses of mean concentrations among sampling sites revealed significant differences existed for arsenic, aluminum, barium, mercury, and selenium. Results are presented in figure 10. Mean concentrations of aluminum and barium were highest at the Verdi sampling site. Aluminum at the Verdi sampling site (51.28 ppm) was not considered significantly higher than the Tracy site (33.4 ppm), but was significantly higher than the Reno sampling site (6.83 ppm). Mean concentrations of arsenic, mercury, and selenium were highest at the Tracy sampling site. Mercury at the Tracy sampling site (0.486 ppm) was not considered higher than the Reno sampling site (0.407 ppm) but was significantly higher than the Verdi sampling site (0.237 ppm).

Verdi

As described previously, aluminum and barium concentrations were highest in rainbow trout compared to brown trout but were likely representative of higher concentrations for the Verdi site. Descriptions of toxicological effects and relevance of mean site concentrations for each constituent are provided below.

Aluminum

Aluminum (Al) in water has been shown to be toxic to fish. Toxicity is dependent on chemical species. Chemical species is primarily determined by pH, although solution contact with solids, concentration of complexing ligands, and temperature will also influence speciation and toxicity (Driscoll et al. 1989). The

mechanism of toxicity varies with pH. Under conditions of low pH (pH < ~ 5), the Al^{+3} ion is the dominant chemical form, although other monomeric forms also occur (Martell and Motekaitis, 1989). Toxic effects may include impaired ion and osmotic regulation, damage to gill epithelium and intracellular Al accumulation causing necrosis and apoptosis of gill ion-transporting cells (Sparling and Lowe, 1996). Under circumneutral conditions (pH 6 - 8), $Al(OH)_3$ becomes the predominant form, and $Al(OH)_3$ will generally precipitate from solution. Within the lower end of this range (pH 5.5 - 6.5), asphyxiation becomes the predominant toxic mechanism. Asphyxiation is believed to be caused by deposition of $Al(OH)_3$ on gill surfaces causing production of excess mucus and inflammation, which may restrict O_2 and CO_2 diffusion across gill membrane (Sparling and Lowe 1996). Observed effects may include increased gill ventilation rates, increased blood CO_2, increased blood lactate, reduced blood O_2, increased gill mucus production, and gill damage. Rapid changes in pH, such as those found in mixing zones of Al-rich acidic waters with higher pH waters, may greatly enhance fish asphyxiation (Witter et al. 1996). Enhanced toxicity may be attributed to Al deposition and polymerization on gill surfaces which form a physical barrier to gaseous exchange. Under more alkaline conditions, (pH>8), aluminate [$Al(OH)_4^-$] becomes the dominant form. Aluminate is soluble, and Al concentrations in solution again increase. The biological availability and toxicity of Al under alkaline conditions are poorly understood (Sparling and Lowe 1996).

The pH levels of the Truckee River prevent aluminum speciation and limit the bioavailability to fish uptake. In addition, aluminum concentrations did not exceed any known adverse biological effect levels. However, the geology of the Truckee River Basin is quite diverse including carbonate, metamorphic, sedimentary, granitic, and volcanic rocks, and unconsolidated deposits derived from the rocks (Covay et al. 1996) which could influence surface water and sediment chemistry and consequently, fish uptake. Data collected by Lawrence (1997) revealed that aluminum concentrations in Truckee River bed-sediment from Farad, California (immediately upstream of Verdi) were six times higher than those downstream (total organic carbon adjusted), providing evidence that site characteristics are likely driving exposures of aluminum to trout. Local anthropogenic sources could also be a factor. According to the EPA Toxics Release Inventory, a metal finishing and processing plant located <1 km from the sampling site released 7,316 and 8,563 pounds of aluminum to the atmosphere in 2000 and 2001, respectively. However, these releases of aluminum were ceased as of 2002.

Barium

The primary source of naturally occurring barium in water results from the leaching and eroding of sedimentary rocks into groundwater (Kojola et al. 1978). Areas immediately upstream of Verdi are comprised of glacial outwash deposits, volcanic mudflow deposits, and quartz monzonite bedrock (Jones and Stokes, Inc. 2002) not of sedimentary origin suggesting that species differences are driving barium concentrations observed in fish. Although barium occurs naturally in most surface water bodies (i.e., approximately 99% of those examined) (Kopp and Kroner 1967), releases of barium to surface waters from natural sources are much

lower than those to groundwater (Kojola et al. 1978). However, barium concentrations did not exceed any known adverse biological effect levels.

Tracy

Concentrations of arsenic, mercury, and selenium were highest among trout at the Tracy sampling site compared to other sites. Descriptions of toxicological effects and relevance of mean site concentrations for each constituent are provided below.

Arsenic

Arsenic (As) in water, sediment, and diet may be toxic to aquatic organisms. Like aluminum, the toxicity of arsenic in water is dependent on chemical species (Eisler 1994). In general, As^{+3} is considered more toxic than As^{+5}. Under oxygenated conditions, formation of As^{+5} would be favored. Arsenic at 1 ppm dry weight is reported to be the 85^{th} percentile background concentration for freshwater fish (Schmitt and Brambaugh 1990). Some fish collected were slightly above, but most were within or below this range and did not exceed other known biological effects or human criteria.

Increases in arsenic in a downstream fashion have been observed in the adjacent terminal basins of the Carson and Walker Rivers but were the result irrigation-induced hydrologic interactions with alluvial sediments (Lico 1992; Lico and Seiler 1994; Welch et al. 1988). Steamboat Hot Springs has been identified as a source of arsenic to the Truckee River via Steamboat Creek (Johannesson et al. 1997) and Steamboat Creek is listed as impaired for arsenic on the State of Nevada's Clean Water Act 303(d) list.

Mercury

As discussed previously, mercury is considered highly toxic, and minor amounts in water, sediment, and diet have been associated with adverse effects to fish and aquatic invertebrates. Mercury in trout at Reno and Tracy did not exceed the t-TEL level determined by Beckvar et al (2005). Nor did trout exceed an avian dietary threshold for reproductive and behavioral effects in loons at 0.3 ppm (Barr 1986). Mercury concentrations in trout were within the Pyramid Lake Paiute Tribal standard for mercury in fish from the Reno site downstream to Tracy.

Although mercury is currently not at a level of concern to implicate negative effects to the fish communities of the Lower Truckee River, the terminal nature of the system could present threats to the fishery at Pyramid Lake due to mercury loads over time. Sources of mercury for fish in the lower Truckee River are likely from the Steamboat Creek drainage. Mercury in Steamboat Creek was originally derived from its headwaters, Washoe Lake, where several gold and silver mills that utilized mercury were located. In the 100 plus years since ore processing occurred, mercury-laden alluvium has been deposited in the stream channel and on streambanks where it is available for remobilization (Blum et al 2001; Stamenkovic et al. 2004). Non-point source urban run-off is also likely providing mercury inputs to fish within the Reno-Sparks urban area but to a lesser degree.

Selenium

Selenium is strongly bioaccumulated in aquatic habitats (Lemly 1996) and relatively low concentrations in water can quickly become concentrated to potentially toxic levels in aquatic organisms. Toxic endpoints commonly include histopathology, physiological effects, reduced reproduction, and reduced survival. Selenium is recognized as a powerful teratogen, and reproduction is generally considered the most sensitive and significant toxic endpoint. However, concentrations did not exceed toxic thresholds of 4 ppm, identified by Lemly (1996). Selenium also did not exceed dietary concentrations associated with reduced juvenile salmonid survival (6.5 - 10 ppm; Lemly 1996). Selenium is also a concern in fish for human consumption. California health officials have established a human consumption criterion of 2.0 µg/g, wet weight, in edible portions of fish and wildlife tissue (Fan et al. 1988). Selenium concentrations were below this criterion for all sites.

Selenium observations may be the result of mobilization from seleniferous soils within Steamboat Creek and Spanish Springs areas via irrigation drainage return flow or from tertiary-treated wastewater effluent. Data from Lawrence (1997) showed increases in selenium for both sediment and crayfish immediately downstream of the Steamboat Creek confluence at Lockwood. However, further studies would be needed to determine and identify the specific sources of selenium.

3.4 Screening of Endocrine Disruption in Trout

Results of hormone concentrations from whole fish samples collected in this study are provided in appendix 3. Mean hormone concentrations determined from salmonid plasma by sampling site and sex are provided in table 11. Two samples (Nos. LTR001FPH007 and LTR001FPH009) were omitted from analysis due to anomalous results likely resulting from laboratory error.

Table 11. Mean hormone concentrations and ratios (by sampling site and sex) collected from blood plasma in trout from the Truckee River, August 2002.

Site	Sex	N	vitellogenin (µg/ml)	17ß-estradiol (ρg/ml)	11-ketotestosterone (ρg/ml)
Verdi					
	Female	4	313.45	649.75	331.50
	Male	4	ND	192.50	534.50
Reno					
	Female	8	448.65	1329.88	405.25
	Male	2	ND	286.50	422.50
Tracy					
	Female	3	230.03	936.67	360.00
	Male	4	4.73	226.00	571.75

Concentrations of vitellogenin (VTG) for individual females among all sites ranged from a low of 8.5 µg/ml to a maximum of 759.2 µg/ml. Concentrations were not detected among most males with the exception of two individuals from the Tracy sampling site at 6.4 and 12.5 µg/ml respectively. However, significant differences were not detected within sexes among sampling sites or species. VTG is a phospholipoglycoprotein manufactured in the liver of mature female fish in response to increasing circulating estrogen levels leading up to spawning (Arukwe et al. 2000). Male fish do not normally produce VTG, but the hepatic estrogen receptor and the gene that encodes for VTG is still present (Maitre et al. 1985; LeGuellec et al. 1988). The result is that when male fish are exposed to estrogenic compounds, VTG production can be induced. In that regard, many studies have reported VTG induction in fish exposed to estrogenic compounds in a laboratory setting (Tyler et al. 1999; Carlson and Williams 1999; Palace et al. 2001a,b; Schultz et al. 2001). Similar results have also been obtained in wild fish captured in freshwaters contaminated with environmental estrogens (Flomar et al. 1996; Harries et al. 1997, 1999; Larsson et al. 1999; Van Aerle et al. 2001). The presence of VTG in the two males combined with the organosomatic data showing immature testes for all male trout collected provides some evidence of potential endocrine disruption in male trout.

Concentrations of 17ß-estradiol for individual females among all sites ranged from a low of 385 ρg/ml to a maximum of 1,615 ρg/ml. Individual male concentrations ranged from a low of 101 ρg/ml to a maximum of 472 ρg/ml. Differences in mean concentrations were not detected for both males and females within each species. Mean estradiol concentrations were also not significantly different between sampling sites for males when species data were combined. However, 17ß-estradiol in combined females was significantly higher at the Reno and Tracy sampling sites compared to Verdi. A number of contaminants have been demonstrated to bind to estrogen receptors from several fish species although typically at several orders of magnitude below that of 17ß-estradiol and synthetic analogues (Loomis and Thomas 1999; Fent 2001; Kloas et al. 2000). Ethynylestradiol is a synthetic estrogen used in oral contraceptives, and its occurrence in surface waters is the result of municipal sewage discharges (Arcand-Hoy and Benson 1998; Larsson et al. 1999). Recently, both 17ß-estradiol and ethynylestradiol have been implicated as the primary contaminants contributing to the estrogenic activity in surface waters from both the United Kingdom and United States (Desbrow et al. 1998; Snyder et al. 1999). The significant differences in females at the Reno and Tracy sites for 17ß-estradiol indicate a potential for elevated estrogenic activity and could indicate endocrine disruption in female trout.

Concentrations of 11-ketotestosterone for individual females among all sites ranged from a low of 65 ρg/ml to a maximum of 645 ρg/ml. Individual male concentrations ranged from a low of 237 ρg/ml to a maximum of 927 ρg/ml. Differences in mean concentrations were not detected among sites for males or females when species were combined. Differences were also not detected in mean concentrations among males within each species. However, concentrations were significantly higher overall for brown trout females (405.27 ρg/ml) compared to their rainbow trout counterparts (297.5 ρg/ml) for all sites combined. Differences observed in 11-ketotestosterone among species, however, would be expected due to temporal differences in spawning periods between brown trout (Fall) and rainbow trout (Spring).

Hileman (1994) concluded that a specific ratio of estrogen to testosterone is necessary for sexual differentiation in developing animals and that alteration of the ratio can result in incomplete or improper gonadal development. Additionally, the balance between these two hormones determines a fish's phenotype, which includes sex characteristics, differentiation of the brain and behavior, and development of other reproductive organs (Lehninger 1982; Hunter and Donaldson 1983). There may be an acceptable range of proportions of female to male sex steroid hormones at various stages in a fishes life cycle, and the range may be most critical in immature and developing fish. In this study, values of the 17ß-estradiol/11KT ratio were below 1.0 for males, and above 1.0 for females. Although ranges have not been established for classifying fish as normal or not, extreme values of the ratio compared to other fish, or correlations between the ratio and contaminant levels, are useful indicators of potential endocrine disruption.

4.0 RECOMMENDATIONS

Perturbations in the health and community structure of fish at sampling sites implicated the cumulative effect of anthropogenic sources on specific reaches in the Truckee River. Results of contaminant concentrations in fish corroborated the results of the health and community assessments. Contaminants identified as accumulating in specific reaches of the Truckee River were PAH in the Reno-Sparks urban area and arsenic, mercury, and selenium in reaches downstream of the Reno-Sparks urban area. Screening of blood plasma hormones also provided evidence that some male trout are being exposed to endocrine disrupting chemicals downstream of the Reno-Sparks urban area, possibly from point source discharges of tertiary-treated sewage effluent.

To reduce potential impacts to fish from non-point urban sources of PAH, existing storm-water drainage systems need to be modified to meet “Low Impact Design” methods currently being implemented by the City of Reno in new urban area developments. PAH entering the river from the Sparks Tanks Farm (a.k.a. Vista Canyon Group Solvent/Fuel Site) needs to be determined and existing cleanup/removal needs to be improved. In addition, restorations of riparian areas and improvements in lotic processes throughout the downtown areas of the river should be implemented to improve biodegradation rates of PAH in the aquatic environment. Opportunities to implement restoration and improve wetland and riparian habitats could be achieved with flood planning activities currently being conducted by the U.S. Army Corps of Enginneers (COE) .

Reductions in uptake and accumulations of metals and trace elements in fish and other aquatic biota can be achieved by improvements of water and sediment quality from wetland and riparian habitat processes. Therefore, creation and restoration of wetland and riparian habitats within the Steamboat Creek drainage should be implemented to help reduce inputs of arsenic, mercury, and selenium to Truckee River downstream of the Reno-Sparks area. Opportunities to restore and improve wetland and riparian habitats could exist if the Service assists and supports planned restoration efforts being conducted by the COE and the Cities of Reno and Sparks. However, creation and restoration of these wetland and riparian habitats may increase methylation rates of mercury and increase risks to biota. Alternatively, if hydrologic regimes in Steamboat Creek could be

consistently maintained, methylation rates could be minimized and long-term risks to fish would be reduced. Such efforts may be achieved if TMWRF discharges were located further upgradient in the Steamboat Creek watershed.

Due to the drought conditions during sample collection, sample sizes were of fish were reduced and therefore limited the statistical strength of analysis for this investigation. This study should be repeated during higher water flows and over several years to validate the limited conclusions observed. Further information also needs to be collected on endocrine disruption in fish downstream of the Reno-Sparks urban area. Information should include: extent of salmonid population impacts; extent of impacts to other species; and identification of endocrine disrupting compounds in sewage discharges and in the river. The potential for controlling contaminants in these discharges may be achieved as TMWRF expands its facilities in the near future.

Until both point and non-point sources of these contaminants are reduced or can be more efficiently managed, long-term successful recovery of Lahontan cutthroat trout may be difficult to achieve in the lower Truckee River.

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Appendix 1. Results of Index of Biotic Integrity scores for Truckee River fish communities and associated metric data for each sample site.

METRIC	Verdi		Reno		Lockwood		Tracy		Marble Bluff	
	Value	Score	Value	Score	Value	Score	Value	Score	Value	Score
Number of native species	4	1	4	0.8	4	0.80	4	0.80	4	0.80
Percent consisting of Paiute sculpin individuals	20.31	1	0	0	0	0	0	0	0	0
Percent consisting of mountain whitefish individuals	4.69	0.47	23.68	1	4.35	0.43	0	0	0	0
Evidence of reproduction by mountain whitefish and/or Paiute sculpin	Yes	1	No	0.5	No	0	No	0	No	0
Percent consisting of Lahontan cutthroat trout individuals	0	0	0	0	0	0	0	0	0	0
Percent of individuals consisting of sensitive species	45.31	1	28.95	1	8.70	0.43	10.53	0.53	0	0
Percent consisting of mountain and/or Tahoe sucker individuals	29.69	0	36.84	0	82.61	0	28.95	0	5.71	0.71
Percent of individuals that are omnivourous as adults	51.56	0	47.37	0	86.96	0	60.53	0	62.86	0
Percent of individuals that are generally tolerant	0	1	10.53	0	0	1	0	1	34.29	0
Percent of individuals that are alien to the Truckee River	53.13	0	63.16	0	4.35	0.83	50	0	34.29	0
Percent of individuals with external anomalies	0	1	0	1	41.30	0	12	0	11.43	0
Total IBI Score	**6.47**		**4.3**		**3.5**		**2.33**		**1.51**	

Appendix 2. Results of polycyclic aromatic hydrocarbon metabolite concentrations in the bile of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- wet weight.

Sample #	Site collected (fig. 1)	Species	sex	fish weight (g)	Benzo(a)pyrene	Naphtalene	Phenanthrene
001FB001	Verdi	rainbow trout	female	392	0.1	39	9.4
001FB002	Verdi	brown trout	male	393	0.3	49	15
001FB003	Verdi	rainbow trout	female	368	0.2	51	18
001FB004	Verdi	rainbow trout	female	402	0.2	26	11
001FB005	Verdi	rainbow trout	male	305	0.4	100	32
002FB01	Reno	brown trout	male	129	0.1	140	49
002FB03	Reno	brown trout	female	132	0.2	73	27
002FB04	Reno	brown trout	female	1115	0.6	190	74
002FB05	Reno	brown trout	female	378	0.4	120	43
004FB01	Tracy	brown trout	female	142	<0.05	3.1	1.5
004FB02	Tracy	brown trout	male	223	0.3	95	37
004FB03	Tracy	brown trout	female	234	0.6	110	42
004FB04	Tracy	brown trout	female	173	0.4	76	30
004FB05	Tracy	brown trout	male	139	0.4	91	39

Appendix 3. Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Aluminum						
-Verdi	243.9	RBT-F	72	4	17	1
	261.4	BNT-M	< 4	4	< 1	1
	243.2	RBT-F	13	4	4.2	1
	343.2	RBT-F	61	4	16	1
	261.4	RBT-M	160	4	36	0.9
	140.3	RBT-F	23	4	6.7	1
	134.4	RBT-M	28	4	7.8	1
-Reno	121.7	BNT-M	8	4	2	1
	102.8	BNT-F	10	4	3	1
	102.5	BNT-F	7	4	2	1
	1210.1	BNT-F	7	4	2	1
	201.8	BNT-F	7	4	2	1
	1265.2	BNT-F	< 4	4	< 1	1
-Tracy	119.9	BNT-M	4	4	1	1
	221.6	BNT-M	10	4	3.2	1
	234.2	BNT-F	74	4	21	1
	181.1	BNT-M	29	4	8.4	1
	141.0	BNT-M	50	4	12	0.9
Arsenic						
-Verdi	243.9	RBT-F	1.4	0.2	0.33	0.05
	261.4	BNT-M	< 0.2	0.2	< 0.06	0.06
	243.2	RBT-F	0.2	0.2	0.07	0.07
	343.2	RBT-F	0.4	0.2	0.1	0.05
	261.4	RBT-M	0.71	0.2	0.17	0.05
	140.3	RBT-F	0.3	0.2	0.08	0.06
	134.4	RBT-M	1.1	0.2	0.3	0.06
-Reno	121.7	BNT-M	0.2	0.2	0.05	0.05
	102.8	BNT-F	< 0.2	0.2	< 0.05	0.05
	102.5	BNT-F	< 0.2	0.2	< 0.05	0.05
	1210.1	BNT-F	< 0.2	0.2	< 0.06	0.06
	201.8	BNT-F	1.7	0.2	0.49	0.06
	1265.2	BNT-F	< 0.2	0.2	< 0.06	0.06
-Tracy	119.9	BNT-M	2.1	0.2	0.5	0.05
	221.6	BNT-M	1.3	0.2	0.36	0.05
	234.2	BNT-F	0.78	0.2	0.22	0.06
	181.1	BNT-M	0.89	0.2	0.26	0.06
	141.0	BNT-M	0.91	0.2	0.21	0.05

Appendix 3 (cont.). Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Barium						
-Verdi	243.9	RBT-F	4	0.2	0.95	0.05
	261.4	BNT-M	0.79	0.2	0.24	0.06
	243.2	RBT-F	4.6	0.2	1.5	0.07
	343.2	RBT-F	4.8	0.2	1.2	0.05
	261.4	RBT-M	6.7	0.2	1.6	0.05
	140.3	RBT-F	1.9	0.2	0.56	0.06
	134.4	RBT-M	1.3	0.2	0.36	0.06
-Reno	121.7	BNT-M	1.4	0.2	0.37	0.05
	102.8	BNT-F	1.3	0.2	0.32	0.05
	102.5	BNT-F	0.78	0.2	0.21	0.05
	1210.1	BNT-F	1.2	0.2	0.39	0.06
	201.8	BNT-F	0.61	0.2	0.2	0.06
	1265.2	BNT-F	0.92	0.2	0.29	0.06
-Tracy	119.9	BNT-M	0.6	0.2	0.1	0.05
	221.6	BNT-M	1.1	0.2	0.3	0.05
	234.2	BNT-F	2.1	0.2	0.61	0.06
	181.1	BNT-M	1	0.2	0.28	0.06
	141.0	BNT-M	0.76	0.2	0.18	0.05
Cadmium						
-Verdi	243.9	RBT-F	0.1	0.1	0.02	0.02
	261.4	BNT-M	< 0.1	0.1	< 0.03	0.03
	243.2	RBT-F	< 0.1	0.1	< 0.03	0.03
	343.2	RBT-F	< 0.1	0.1	< 0.03	0.03
	261.4	RBT-M	< 0.1	0.1	< 0.02	0.02
	140.3	RBT-F	0.2	0.1	0.05	0.03
	134.4	RBT-M	0.3	0.1	0.08	0.03
-Reno	121.7	BNT-M	0.98	0.1	0.26	0.03
	102.8	BNT-F	< 0.1	0.1	< 0.03	0.03
	102.5	BNT-F	0.3	0.1	0.07	0.03
	1210.1	BNT-F	< 0.1	0.1	< 0.03	0.03
	201.8	BNT-F	0.1	0.1	0.04	0.03
	1265.2	BNT-F	< 0.1	0.1	< 0.03	0.03
-Tracy	119.9	BNT-M	0.3	0.1	0.064	0.02
	221.6	BNT-M	0.1	0.1	0.03	0.03
	234.2	BNT-F	< 0.1	0.1	< 0.03	0.03
	181.1	BNT-M	< 0.1	0.1	< 0.03	0.03
	141.0	BNT-M	0.2	0.1	0.05	0.02

Appendix 3 (cont.). Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Chromium						
-Verdi	243.9	RBT-F	0.8	0.5	0.2	0.1
	261.4	BNT-M	< 0.5	0.5	< 0.2	0.2
	243.2	RBT-F	< 0.5	0.5	< 0.2	0.2
	343.2	RBT-F	1	0.5	0.34	0.1
	261.4	RBT-M	1	0.5	0.3	0.1
	140.3	RBT-F	< 0.5	0.5	< 0.1	0.1
	134.4	RBT-M	1.6	0.5	0.45	0.1
-Reno	121.7	BNT-M	0.8	0.5	0.2	0.1
	102.8	BNT-F	< 0.5	0.5	< 0.1	0.1
	102.5	BNT-F	7.1	0.5	1.9	0.1
	1210.1	BNT-F	0.5	0.5	0.2	0.2
	201.8	BNT-F	< 0.5	0.5	< 0.1	0.1
	1265.2	BNT-F	< 0.5	0.5	< 0.2	0.2
-Tracy	119.9	BNT-M	1	0.5	0.33	0.1
	221.6	BNT-M	< 0.5	0.5	< 0.5	0.1
	234.2	BNT-F	< 0.5	0.5	< 0.1	0.1
	181.1	BNT-M	0.7	0.5	0.2	0.1
	141.0	BNT-M	0.6	0.5	0.1	0.1
Copper						
-Verdi	243.9	RBT-F	8.2	0.3	2	0.07
	261.4	BNT-M	4.9	0.3	1.5	0.09
	243.2	RBT-F	4.6	0.3	1.5	0.1
	343.2	RBT-F	8.7	0.3	2.3	0.08
	261.4	RBT-M	5.1	0.3	1.2	0.07
	140.3	RBT-F	5.2	0.3	1.5	0.09
	134.4	RBT-M	27	0.3	7.6	0.08
-Reno	121.7	BNT-M	5.4	0.3	1.4	0.08
	102.8	BNT-F	3.4	0.3	0.84	0.08
	102.5	BNT-F	15	0.3	4.1	0.08
	1210.1	BNT-F	6.5	0.3	2	0.09
	201.8	BNT-F	14	0.3	4	0.09
	1265.2	BNT-F	9.8	0.3	3.1	0.09
-Tracy	119.9	BNT-M	12	0.3	2.9	0.07
	221.6	BNT-M	6.9	0.3	1.8	0.08
	234.2	BNT-F	6.3	0.3	1.8	0.09
	181.1	BNT-M	8.8	0.3	2.5	0.09
	141.0	BNT-M	4.6	0.3	1.1	0.07

Appendix 3 (cont.). Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Iron						
-Verdi	243.9	RBT-F	110	2	27	0.5
	261.4	BNT-M	22	2	6.7	0.6
	243.2	RBT-F	45	2	15	0.7
	343.2	RBT-F	120	2	30	0.5
	261.4	RBT-M	343	2	80.2	0.5
	140.3	RBT-F	60	2	18	0.6
	134.4	RBT-M	71	2	20	0.6
-Reno	121.7	BNT-M	29	2	7.7	0.5
	102.8	BNT-F	35	2	8.8	0.5
	102.5	BNT-F	74	2	20	0.5
	1210.1	BNT-F	45	2	14	0.6
	201.8	BNT-F	41	2	12	0.6
	1265.2	BNT-F	41	2	13	0.6
-Tracy	119.9	BNT-M	37	2	9.1	0.5
	221.6	BNT-M	41	2	11	0.5
	234.2	BNT-F	88	2	25	0.6
	181.1	BNT-M	72	2	21	0.6
	141.0	BNT-M	73	2	17	0.5
Mercury						
-Verdi	243.9	RBT-F	0.2	0.1	0.04	0.02
	261.4	BNT-M	0.2	0.1	0.06	0.03
	243.2	RBT-F	0.3	0.1	0.093	0.03
	343.2	RBT-F	0.42	0.1	0.11	0.03
	261.4	RBT-M	0.2	0.1	0.06	0.02
	140.3	RBT-F	0.2	0.1	0.06	0.03
	134.4	RBT-M	0.1	0.1	0.03	0.03
-Reno	121.7	BNT-M	0.3	0.1	0.07	0.03
	102.8	BNT-F	0.3	0.1	0.07	0.03
	102.5	BNT-F	0.37	0.1	0.098	0.03
	1210.1	BNT-F	0.78	0.1	0.25	0.03
	201.8	BNT-F	0.1	0.1	0.04	0.03
	1265.2	BNT-F	0.59	0.1	0.18	0.03
-Tracy	119.9	BNT-M	0.51	0.1	0.12	0.02
	221.6	BNT-M	0.45	0.1	0.12	0.03
	234.2	BNT-F	0.45	0.1	0.13	0.03
	181.1	BNT-M	0.48	0.1	0.14	0.03
	141.0	BNT-M	0.54	0.1	0.13	0.02

Appendix 3 (cont.). Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Magnesium						
-Verdi	243.9	RBT-F	1260	2	299	0.5
	261.4	BNT-M	979	2	295	0.6
	243.2	RBT-F	804	2	263	0.7
	343.2	RBT-F	1220	2	315	0.5
	261.4	RBT-M	1460	2	340	0.5
	140.3	RBT-F	1040	2	305	0.6
	134.4	RBT-M	1080	2	305	0.6
-Reno	121.7	BNT-M	1080	2	289	0.5
	102.8	BNT-F	1380	2	345	0.5
	102.5	BNT-F	1140	2	305	0.5
	1210.1	BNT-F	1010	2	317	0.6
	201.8	BNT-F	1140	2	328	0.6
	1265.2	BNT-F	1060	2	334	0.6
-Tracy	119.9	BNT-M	1180	2	287	0.5
	221.6	BNT-M	1130	2	303	0.5
	234.2	BNT-F	1120	2	323	0.6
	181.1	BNT-M	1120	2	319	0.6
	141.0	BNT-M	1320	2	312	0.5
Manganese						
-Verdi	243.9	RBT-F	9.2	0.5	2.2	0.1
	261.4	BNT-M	3.1	0.5	0.94	0.2
	243.2	RBT-F	8.6	0.5	2.8	0.2
	343.2	RBT-F	5.3	0.5	1.4	0.1
	261.4	RBT-M	11	0.5	2.6	0.1
	140.3	RBT-F	6.1	0.5	1.8	0.1
	134.4	RBT-M	3.5	0.5	0.98	0.1
-Reno	121.7	BNT-M	3.3	0.5	0.89	0.1
	102.8	BNT-F	6.6	0.5	1.7	0.1
	102.5	BNT-F	4.5	0.5	1.2	0.1
	1210.1	BNT-F	3.9	0.5	1.2	0.2
	201.8	BNT-F	4.3	0.5	1.2	0.1
	1265.2	BNT-F	4.2	0.5	1.3	0.2
-Tracy	119.9	BNT-M	1.6	0.5	0.4	0.1
	221.6	BNT-M	5	0.5	1.3	0.1
	234.2	BNT-F	7.8	0.5	2.2	0.1
	181.1	BNT-M	4.7	0.5	1.3	0.1
	141.0	BNT-M	3.9	0.5	0.92	0.1

Appendix 3 (cont.). Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Selenium						
-Verdi	243.9	RBT-F	1.1	0.3	0.26	0.07
	261.4	BNT-M	0.4	0.3	0.1	0.09
	243.2	RBT-F	0.3	0.3	0.1	0.1
	343.2	RBT-F	0.7	0.3	0.2	0.08
	261.4	RBT-M	0.9	0.3	0.2	0.07
	140.3	RBT-F	0.4	0.3	0.1	0.09
	134.4	RBT-M	0.9	0.3	0.24	0.08
-Reno	121.7	BNT-M	0.6	0.3	0.2	0.08
	102.8	BNT-F	0.6	0.3	0.1	0.08
	102.5	BNT-F	0.4	0.3	0.1	0.08
	1210.1	BNT-F	0.8	0.3	0.2	0.09
	201.8	BNT-F	1.2	0.3	0.35	0.09
	1265.2	BNT-F	0.7	0.3	0.2	0.09
-Tracy	119.9	BNT-M	1.2	0.3	0.3	0.07
	221.6	BNT-M	1.3	0.3	0.34	0.08
	234.2	BNT-F	1.1	0.3	0.32	0.09
	181.1	BNT-M	1	0.3	0.29	0.09
	141.0	BNT-M	1.3	0.3	0.3	0.07
Strontium						
-Verdi	243.9	RBT-F	25.8	0.2	6.14	0.05
	261.4	BNT-M	21.8	0.2	6.55	0.06
	243.2	RBT-F	33.8	0.2	11.1	0.07
	343.2	RBT-F	41.7	0.2	10.8	0.05
	261.4	RBT-M	37.4	0.2	8.73	0.05
	140.3	RBT-F	23.2	0.2	6.8	0.06
	134.4	RBT-M	8.9	0.2	2.5	0.06
-Reno	121.7	BNT-M	20.1	0.2	5.39	0.05
	102.8	BNT-F	34.5	0.2	8.65	0.05
	102.5	BNT-F	21.6	0.2	5.78	0.05
	1210.1	BNT-F	36.7	0.2	11.5	0.06
	201.8	BNT-F	22.5	0.2	6.47	0.06
	1265.2	BNT-F	41.2	0.2	13	0.06
-Tracy	119.9	BNT-M	15	0.2	3.7	0.05
	221.6	BNT-M	19	0.2	5.22	0.05
	234.2	BNT-F	26.9	0.2	7.75	0.06
	181.1	BNT-M	21.9	0.2	6.26	0.06
	141.0	BNT-M	21.1	0.2	5	0.05

Appendix 3 (cont.). Results of metals and trace element concentrations in the whole bodies of trout collected from sites on the Truckee River, August 2002. Results are reported in parts per million- dry weight. (RBT= rainbow trout; BNT=brown trout; F=female; M=male)

Analyte -Location	Fish weight (g)	Species-Sex	Dry Weight (ppm)	Detection Limit (dry)	Wet Weight (ppm)	Detection Limit (wet)
Zinc						
-Verdi	243.9	RBT-F	164	0.5	39.1	0.1
	261.4	BNT-M	72.2	0.5	21.7	0.2
	243.2	RBT-F	88.1	0.5	28.9	0.2
	343.2	RBT-F	126	0.5	32.7	0.1
	261.4	RBT-M	139	0.5	32.5	0.1
	140.3	RBT-F	95.6	0.5	27.9	0.1
	134.4	RBT-M	149	0.5	42.1	0.1
-Reno	121.7	BNT-M	112	0.5	29.9	0.1
	102.8	BNT-F	150	0.5	37.6	0.1
	102.5	BNT-F	101	0.5	27	0.1
	1210.1	BNT-F	112	0.5	35.2	0.2
	201.8	BNT-F	136	0.5	39.1	0.1
	1265.2	BNT-F	117	0.5	36.7	0.2
-Tracy	119.9	BNT-M	167	0.5	40.5	0.1
	221.6	BNT-M	122	0.5	32.7	0.1
	234.2	BNT-F	165	0.5	47.5	0.1
	181.1	BNT-M	175	0.5	49.8	0.1
	141.0	BNT-M	135	0.5	32	0.1

Appendix 4. Results of hormone concentrations in blood plasma collected from trout at sampling sites on the Truckee River, August 2002 (vitellogenin= VTG; 17-β estradiol= E2; 11-ketotestosterone=11KT).

Sample Site (fig. 1)	Sample Number	Species	Sex	VTG (ug/ml)	E2 (pg/ml)	11KT (pg/ml)
Reno						
	LTR002FPH01	Brown Trout	M	0.0	101	340
	LTR002FPH02	Brown Trout	F	22.0	652	341
	LTR002FPH03	Brown Trout	F	54.0	853	318
	LTR002FPH04	Brown Trout	F	759.2	1561	395
	LTR002FPH05	Brown Trout	F	718.8	1461	446
	LTR002FPH06	Brown Trout	F	442.3	1615	645
	LTR002FPH07	Brown Trout	F	738.8	1456	386
	LTR002FPH08	Brown Trout	F	638.3	1582	384
	LTR002FPH09	Rainbow Trout	F	215.8	1459	327
	LTR002FPH10	Brown Trout	M	0.0	472	505
Tracy						
	LTR004FPH01	Brown Trout	M	6.4	143	420
	LTR004FPH02	Brown Trout	F	503.3	1418	621
	LTR004FPH03	Brown Trout	F	178.3	1007	394
	LTR004FPH04	Brown Trout	M	12.5	441	806
	LTR004FPH05	Brown Trout	M	0.0	120	401
	LTR004FPH06	Brown Trout	F	8.5	385	65
	LTR004FPH07	Brown Trout	M	0.0	200	660
Verdi						
	LTR001FPH01	Rainbow Trout	F	484.4	536	199
	LTR001FPH02	Brown Trout	M	0.0	246	927
	LTR001FPH03	Rainbow Trout	F	218.0	627	315
	LTR001FPH04	Rainbow Trout	F	22.2	901	349
	LTR001FPH05	Rainbow Trout	M	0.0	237	410
	LTR001FPH06	Brown Trout	F	529.2	535	463
	LTR001FPH07	Rainbow Trout	M	9.8	NS	NS
	LTR001FPH08	Rainbow Trout	M	0.0	134	237
	LTR001FPH09	Rainbow Trout	F	53509	1035	52
	LTR001FPH010	Rainbow Trout	M	0.0	153	564